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CHICKEN-QUAIL HYBRIDS
(See page 167)

Pugnose Mice

Hydrocephalus in Cattle

Endomitosis in Polyploid Fern Prothalli

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NORMAL AND HYDROCEPHALIC COMPARED

Figure 1

Comparison of normal (*A*) and hydrocephalic (*B*) ventricles within the cerebrum of the calf.

THE INHERITANCE OF HYDROCEPHALUS IN CATTLE

MARVEL L. BAKER, LOYAL C. PAYNE AND GUY N. BAKER*

MANY references to hydrocephalus in mammalian life including man are found in the literature. Many of these are clinical reports or case studies. In 1959, Blackwell *et al.*[1] reported the occurrence of a hydrocephalic lethal in the beef cattle herd of the New Mexico Agricultural Experiment Station and included a brief review of the literature, especially that concerning calves. They ascribed the occurrence of the lethal to a single recessive gene.

Kobozieff and associates[2], in reporting their work with mice, also give a rather extensive bibliography including a number of references to hydrocephalus in cattle as well as in other domestic and laboratory animals.

Hydrocephalus in cattle in Nebraska was first called to the attention of the senior author 15 or 20 years ago by a breeder of purebred cattle. In the intervening years it was reported from time to time by commercial producers in Nebraska in all of the major beef breeds. It also was reported in a few purebred herds.

By 1957 the occurrence of hydrocephalic calves had attracted keen interest and concern on the part of producers who were reporting losses among their calves frequently as high as two percent and sometimes higher.

Commercial producers who made a practice each year of breeding replacement heifers to newly purchased bulls reported hydrocephalic calves from the heifers year after year, indicating that if hydrocephalus was genetic in nature, it was fairly widespread.

Field observations and available pedigree information indicated that the hydrocephalus occurring among calves in Nebraska was hereditary in origin. However, it was considered desirable to investigate its nature and evaluate the hypothesis that the condition was inherited as a simple autosomal recessive.

Materials and Methods

Through the cooperation of interested commercial producers a herd of 40 cows known to have produced hydrocephalic calves was assembled. One of these breeders was able to provide a bull (designated W) known to have sired such calves. Twenty of the cows were mated to this bull in 1958 and the other half to him in 1959. Each year the remaining cows were mated to a University-bred bull (designated U) from an inbred line with no history of hydrocephalus. In addition some information and data were obtained from purebred breeders and were utilized as additional material.

Gross examinations were made on the heads of 22 calves exhibiting definite hydrocephalus. Eight living hydrocephalic calves were also studied. The following techniques were used in this study: Some of the heads were placed in a low-temperature freezer (—20° C.) for several days and then examined by serial sectioning with a bone saw. Some heads were submerged in seven percent formalin solution for gross dissection. The left lateral ventricle of the cerebrum of other specimens was injected with a saturated solution of FD and C #1 green stain or "Jeltrate," a fast setting plastic. Cerebro-ventricular and spinal fluid pressures

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were determined by inserting an 18-gauge hypodermic needle into the ventricle or subarachnoid space and allowing the fluid to rise in a clear plastic tube. Bone studies were made using the standard sodium hydroxide-hydrogen peroxide maceration method.

Results

Thirty-two calves were sired by sire W. Sixteen of these were born in 1959 and an equal number in 1960. Four of the calves born in 1959 and two in 1960 were hydrocephalic.

Thirty-seven normal and no hydrocephalic calves were sired by sire U. Nineteen of these were born in 1959 and 18 in 1960.

In 1959 the first hydrocephalic calf was born four days after the first calf in the group, and the last one six days before the last calf was born. In 1960 one hydrocephalic and one normal calf were born on the same day, the first two calves in the group. The second hydrocephalic calf was born eight days later and 20 days before the last calf in the group. Reports from some cattlemen suggest that all hydrocephalic calves come early in the season. Our data do not support this although data on differences in gestation length are not available. The hydrocephalic calves were lighter in weight at birth than the normal calves. For the two years the hydrocephalic calves averaged 52.4 pounds and the normal calves by the same sire 66.3 pounds. In 1959 two of the hydrocephalic calves were male and two were female. In 1960, one calf was a female; the sex of the second calf was not recorded.

In a purebred herd studied, 32 calves were produced from cows previously identified as having produced hydrocephalic calves. Of the 32 calves produced from these cows and sired by bulls that had previously sired hydrocephalic calves, 23 were normal and nine were hydrocephalic.

Thus the following data are available with an expected ratio of three normal to one hydrocephalic calf under the hypothesis that the condition is inherited as a simple autosomal recessive:

Source of Data	*Experimental Year*	*Adjusted Chi-square*
North Platte	1959 calves 12:4	0.0
North Platte	1960 calves 14:2	.75
Purebred herd	23:9	.04

In addition, the reproductive performance of other purebred cattle was considered, the data being made available by the breeders concerned.

A group of 107 cows sired by three bulls identified as sires of hydrocephalic calves were bred to four bulls also identified as sires of such calves for a total of 325 possible calvings. Data for actual calvings were not obtained, only for cows exposed to the bulls and for hydrocephalic calves noted.

From the total of 325 matings, 31 hydrocephalic calves were produced, as shown in Table I.

The expected frequency of heterozygous cows in the 107-cow group would be between 0.5 and 0.67 if selection were directed only against the abnormal calves. We would then expect between 41 and 54 hydrocephalic calves, assuming that a 100 percent calf crop was obtained, or 35 and 46 hydrocephalic calves with an 85 percent calf crop. As pointed out, the number of cows actually producing calves was not obtained. In addition to cows not conceiving, some bred females were sold.

In a fourth group where a bull was bred to five of his daughters, two hydrocephalic calves were produced.

The results of the gross examinations of the heads of 22 calves hydrocephalic

TABLE I. Calvings by sires

Calf groups by sires	No. matings	Calves	
		Normal	Hydrocephalic
E	17	15*	2
F	73	68*	5
G	154	134*	20
H	81	77*	4
Sire X daughters	5	3	2

*Data for normal calves assume 100 percent calf crops; the data obtained were for cows bred and hydrocephalic calves produced.

and of eight living calves may be summarized as follows: there is a direct relationship between the amount of cranial enlargement, ventricular size, volume of cerebro-ventricular fluid and locomotive incoordination. The inability of the newborn calf to stand and nurse results in the various names which have been given to this disturbance, "bawlers," "dummies," and "bulldogs."

Examination of the bony sutures of the cranium (frontal, nasofrontal, parietal, etc.) indicates a normal and complete closure of these fissures. In none of the heads examined was there any gross evidence of separation of these sutures.

Examination of the brain and upper spinal cord by gross dissection of the frozen formalinized specimen, the injection of fast green dye or plastic into the lateral ventricle, or the gravitation of dye into the ventricle and the subsequent detection of this dye in a hypodermic needle inserted into the subarachnoid space of the spinal cord, indicated there was no obstruction in the communications of this system. In all specimens, either living or dead, there was a free flow of the injected material from the ventricle into the cerebral and spinal arachnoidea.

The most obvious anatomical change seen during these dissections (other than the greatly enlarged cerebral ventricles and the destruction of the cerebral medulla and cortex, Figure 1) was the marked enlargement of the choroid plexus. In the extreme hydrocephalic animal there was a four to five-fold increase in the size of this structure over that of the normal calf. Whether this change was "cause or result" was not determined.

In the living animal, one to three days of age, cerebral and spinal pressures measured from within the ventricle or at the dorsal opening of the atlantooccuptal articulation fell within normal range of 0-10 millimeters water pressure.

Observations of the lumbar vertebrae, measurement of the heart size, and attempts to relate the occurrence of hydrocephalus with dwarfism were made by observing the lumbar vertebrae formation on X-ray plates, measurement of the heart, determination of cerebrospinal fluid pressures, and detection of orbitosphenoid projections. In all of the specimens studied, there was no evidence of any of the characteristics of dwarfism in these animals.

Discussion

Reports from producers and our own observations indicate that affected calves are usually born alive. However, they are weak, and make little effort to stand. After floundering weakly for a time they lie on the ground with the feet close to the body. The head is not held in a normal position. Frequently it is lower than normal with the ears drooping. Sometimes it is thrown back along the calf's side. The calves emit a characteristic "bawl" and frequently are referred to as "bawlers." As they become weaker they assume a prone position.

No case of a calf standing or nursing, even when helped, has come to the attention of the authors. They usually die within a short time following birth but may live for from 24 to 48 hours. The few reported attempts to rear them by feeding have been unsuccessful. At most, they have prolonged life by only a few hours.

The forehead frequently is noticeably bulged, the skull dome-shaped, the eyes small and the eye-socket reduced in size. The lower jaw frequently is light, tapering to a narrow muzzle. The teeth frequently are poorly developed. Whether calves are born prematurely is not established by available data. Calving usually is accompanied by the discharge of an excessive amount of amniotic fluid and some breeders mark the calves as "drowned" on calving records.

Limited pedigree studies suggest that if hydrocephalus is indeed due to a single gene, the gene occurs widely. No attempt has been made to estimate its frequency in the cattle population at large.

That the gene frequency may become relatively high in individual herds is of course apparent. For example, in one purebred herd of more than 50 cows in 1960, only one was not sired by a known sire of hydrocephalic calves. In another somewhat larger herd in which hydrocephalus has occurred for at least 20 years the frequency of the gene must necessarily be high. One commercial producer, who has depended almost exclusively for many years upon this purebred herd for bulls, reports that from 500 cows bred for 1960 calvings, 25 hydrocephalic calves were produced. This indicates a relatively high gene frequency, with at least 20 percent and a

likelihood of as many as 44 percent of the cows heterozygous for hydrocephalus.

Neither of these herds has any history of dwarfism; nor is there any indication from our work or observations that dwarfism and hydrocephalus are a part of the same syndrome.

The gross examination of hydrocephalic heads and studies of living calves give little insight as to the cause of hydrocephalus in calves. It is obvious that this type of disturbance is not typical of the "internal obstructive hydrocephalus" described in the texts of human pathology.

The inheritance of this disturbance and the genetic relationship seem self-evident from the foregoing genetic studies. The actual cause or course of development of hydrocephalus remains obscure. The measurement of cerebrospinal fluid pressures subsequent to birth indicates that at this time, there is no marked elevation of these pressures. However, this does not indicate what these pressures have been during the embryological development of the animal. The complete and normal closure of the fissures of the bones of the cranium indicates that if highly elevated intraventricular pressures are responsible for hydrocephalus, these pressures must have occurred early in the developmental stage of the fetus. A search of the literature fails to give adequate information as to the embryological development of the cranium of the calf. Hence, it is difficult to assign any given time in the embryonic stage during which hydrocephalus develops.

It should be emphasized that all of these studies are postnatal observations. Future studies concerned with the development of hydrocephalus must be based on observations made during the embryonic development of the calf.

Summary and Conclusions

Forty cows identified as producers of hydrocephalic calves were used in experimental matings in each of two years. Each year, 20 of the cows were bred to a bull identified as a sire of hydrocephalic calves and 20 to a bull from an inbred line with no history of hydrocephalus. During the two years each bull was bred to both groups of cows. Extensive data also were obtained from purebred breeders.

Data from these experimental matings and from the purebred breeders' herds support the hypothesis that the hydrocephalic condition in calves widely reported in Nebraska and from several other states is inherited and that a single autosomal recessive gene is the basis of its inheritance.

The gross examinations of the hydrocephalic heads indicate that the condition is a result of a malformation of the cranium which occurs in early embryonic or fetal development. It is not the type of "congenital hydrocephalus" which is due to an obstruction of the drainage of cerebrospinal fluid from the ventricles. Inasmuch as hydrocephalus is well established at the time of birth, any studies concerned with the mechanism of development must be made during the embryonic or fetal stages of growth.

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ENDOMITOSIS IN A POLYPLOID SERIES OF FERN PROTHALLI

CARL R. PARTANEN*

THE occurrence of somatic polyploidy, or endopolyploidy, has by now been recognized in various somatic cells in a wide variety of plant species[2, 4, 14]. The polyploidy is arrived at through a process variously referred to as "endomitosis," "endomitotic reduplication" or "endoreduplication." The term "endomitosis" was first applied by Geitler to the process as observed in *Gerris lateralis*[3], and the original definition included certain distinctive visible occurrences in the nucleus, i.e., contraction and separation of chromosomes. Later, others also observed polyploidy which arose during the interphase, but without some of the visible events which had been included in Geitler's original description. Hence, other terms were applied to distinguish between the processes. However, Geitler[4] has subsequently recognized that despite the presence or absence of such visible phenomena the process is essentially the same wherever it occurs, and has therefore broadened his original definition to include all such polyploidization occurring during the interphase. Indeed, the crucial point is the occurrence of more than one chromosome replication cycle without the formation and functioning of a mitotic apparatus. Further, with the recognition of species differences in the process, especially with respect to the timing of the optically visible separation of the replicated units[2, 4], it would seem that the term "endomitosis" can be applied to the general process, rather than be reserved for a specific case. Distinctions based upon visible criteria at or near the limits of resolution of the optical system are extremely arbitrary and, as in this case, may be limiting to the overall concept. Perhaps the term "endomitosis" is unfortunate since it can imply a visible physical event. However, the broader, more fundamental implication is that of the additional replication of chromosomes without the occurrence of true mitosis, resulting eventually in more chromosomes, whether they be united or separated. If a distinction must be made between the various degrees of physical separation of the replicated chromosome strands, the terms "polyteny" and "polyploidy" can be used. Hence, the term "endomitosis," as used here, shall refer to the general case of one or more additional replications of the chromosomes during a single interphase.

Since most of the observations of endomitosis in plants have been made in angiosperms, these observations have been almost entirely upon the sporophytic phase of the life cycle, the gametophytic phase having been reduced to a near minimum. Polyploidy, of course, plays an important role in the female gametophyte (the embryo sac), but this is generally arrived at through fusion of nuclei. Since the gametophytic phase of the angiosperm is rather brief and greatly reduced, there would seem to be relatively little opportunity for the occurrence of endomitosis. There are relatively few "somatic" nuclei, and since it is axiomatic that endomitosis does not occur in those cells which are naturally in the direct lineage, i.e., the "germ line," it would seem relatively improbable that endomitosis be encountered there. There are, however, some reports of endomitosis in embryo sac nuclei

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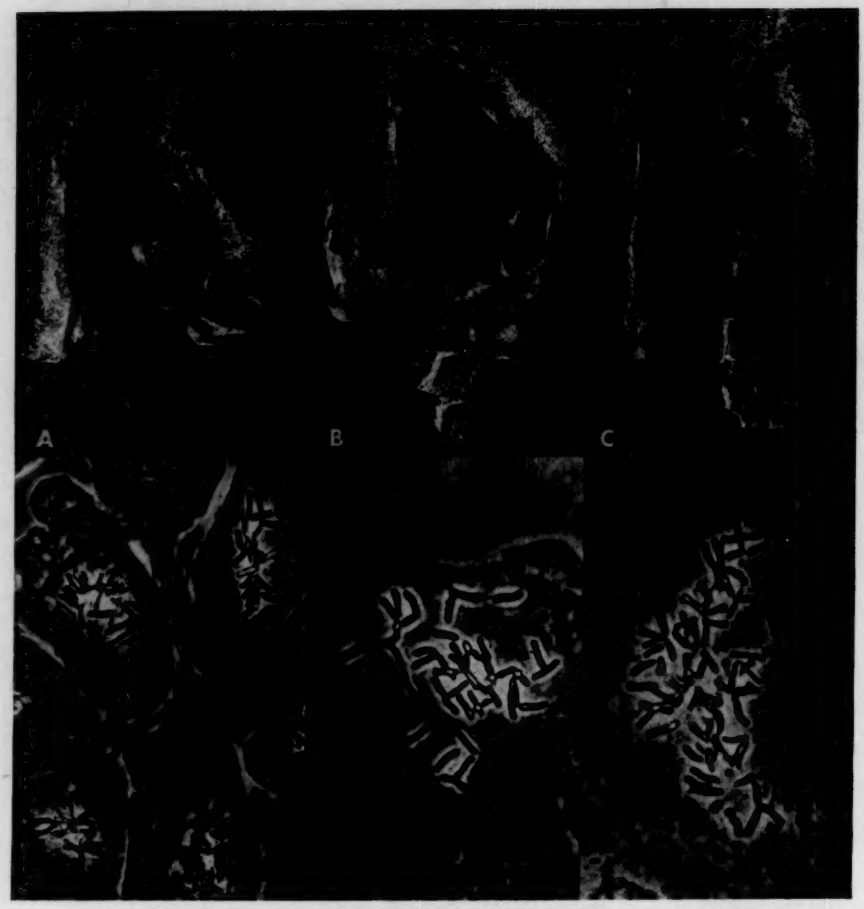


POLYPLOID SERIES IN PROTHALLI OF *OSMUNDA CINNAMOMEA*

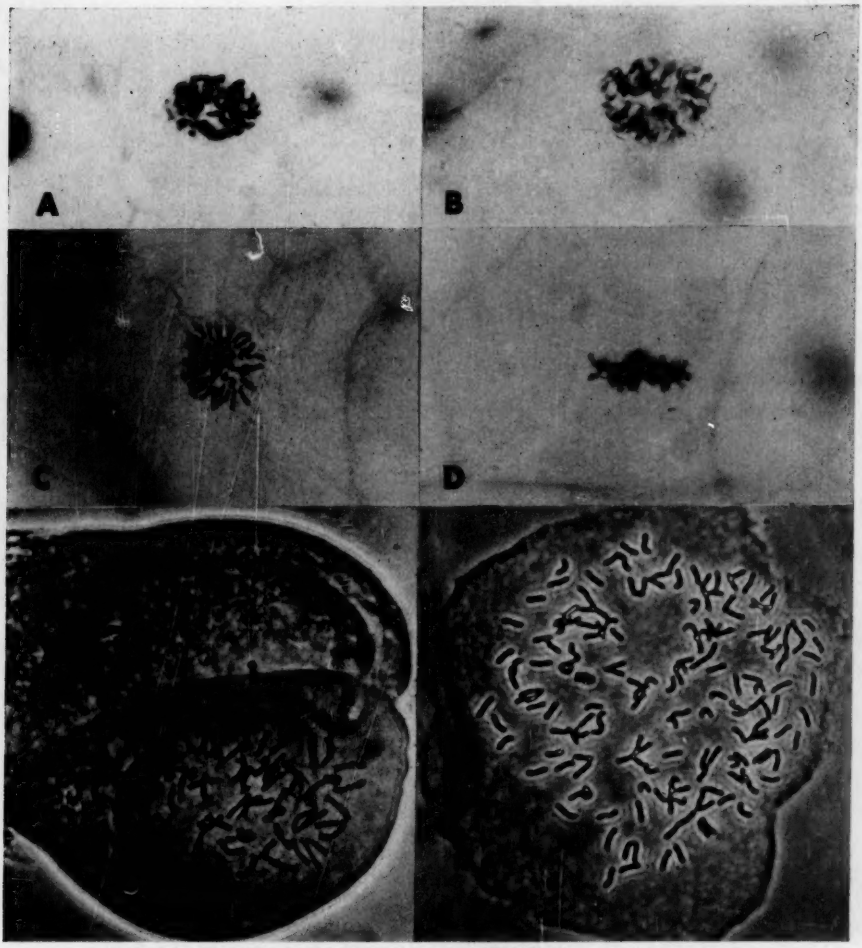
Figure 2

A—Haploid. *B*—Diploid. *C*—Tetraploid (scale for all = mm). *D* to *F*—Corresponding metaphase chromosomes (ca. 850×).

other than the egg nucleus[1].

However, in the ferns, in which the gametophytic generation is a separate entity of a more complex morphology and longer duration, the occurrence of endomitosis could be expected to be more frequent, simply on the basis of cell numbers, duration, and diversification, if these be factors involved. We had earlier observed the occasional occurrence of endomitotic polyploidy in fern prothalli after treatment with the growth hormone indoleacetic acid (IAA)[12], and of higher than 2C (duplicated haploid) levels of desoxyribonucleic acid (DNA) in individual nuclei of untreated prothalli[9]. The potentiality for endomitosis was found to be expressed to a much greater extent in tumors isolated from fern prothalli, as a secondary characteristic of the tumorous condition.

The purpose of the present investi-



ENDOMITOSIS IN PROTHALLI OF *OSMUNDA CINNAMOMEA*
Figure 3

A—Normal prophase in diploid. *B*—Post-endomitotic prophase in diploid, showing diplo-chromosomes. *C*—Polar view of post-endomitotic metaphase in diploid, showing pairs of separated diplo-chromosomes. *D*—Equatorial view, showing simultaneous splitting of diplo-pairs. *E*—Metaphase showing chromatid separation at start of centromere separation. *F*—8*n* metaphase in 4*n* prothallus. (All figures ca. 650×.)

gation was two-fold: to investigate further the occurrence of endomitosis in prothallial nuclei and to determine whether the initial level of ploidy had any effect on the extent and degree of endomitosis. Consequently, comparative cytological and cytophotometric studies were made on haploid, diploid and tetraploid clonal strains of the gametophytes of *Osmunda cinnamomea*.

Materials and Methods

All gametophytes used in these studies were grown aseptically *in vitro* on a chemically defined medium which consisted of essentially a Knudson's mineral salt solution with added minor elements as previously described[13], with 0.25 percent sucrose and 1.5 percent Difco Bacto-agar added. The original haploid gametophyte cultures were started from field-

collected spores of *Osmunda cinnamomea*. The polyploid gametophytes were obtained from this original strain by induced apospory[6]. When a sexual sporeling (diploid) appeared in a gametophyte culture, its leaves were detached and placed individually upon the same type of medium in separate culture tubes. Some of these proliferated gametophytic tissue, which was then isolated and continued in culture as gametophytes. Eventually a sexual sporeling appeared in one of these diploid gametophyte cultures, and through a repetition of the process a tetraploid gametophyte culture was established. All of these strains have been carried in continuous culture now for six years.

Divisions in post-endomitotic nuclei were induced by placing the prothalli in a 10 ppm solution of IAA for 48 hours. The prothalli were then fixed in absolute alcohol : acetic acid (3:1), hydrolyzed in 1N HCl for about 15 minutes, squashed in aceto-carmine and examined in phase contrast.

The haploid, diploid, and tetraploid gametophytes were taken, untreated, from stock cultures for photometric measurements of DNA in individual nuclei. These were fixed, embedded, sectioned, mounted, hydrolyzed, and Feulgen stained as previously described[9]. The sections of each were cut sufficiently thick to accommodate the largest nuclei in sufficient numbers. All preparations for this study were processed at the same time and a haploid prothallus was carried on each slide as a standard.

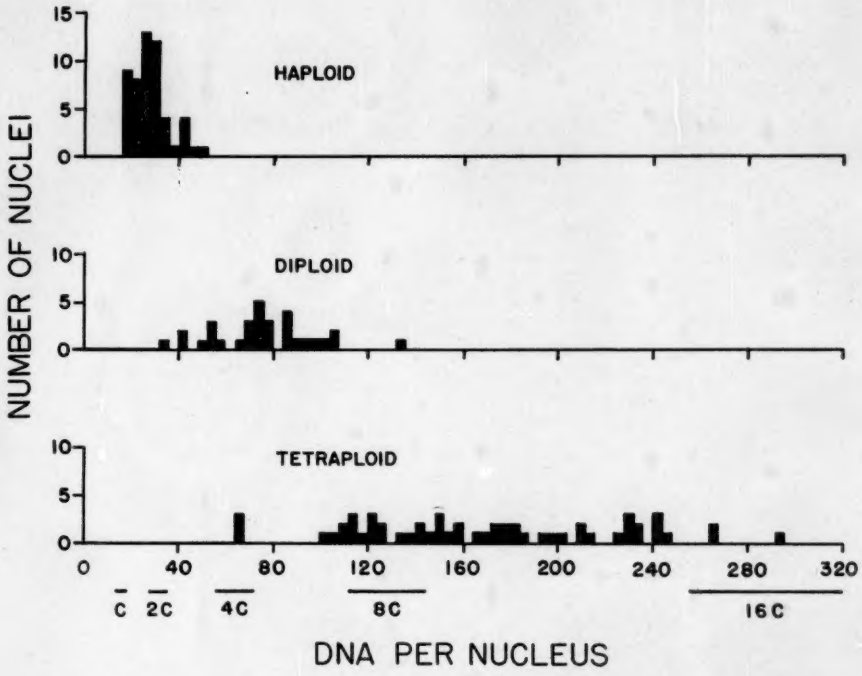
The photometric measurements were made by the two-wavelength method[8], using simplified calculation tables[7]. The microspectrophotometer includes the following components: a battery-fed ribbon filament light source, a Perkin-Elmer single pass prism monochromator; an optical system with a 5× N.A. 0.15 relay lens, 10× N.A. 0.30 condenser lens, 60× N.A. 1.32 objective (all objectives apochromatic), and a 5× compensating ocular. The photometer head employs the sliding prism principle with three positions: sighting telescope, camera, and phototube. The tube response was extremely linear in relation to aperture size, the light beam being focused by a lens onto the end-on tube. Hence, the limiting aperture was photometrically calibrated. The detector unit employs an RCA 6217 multiplier phototube and an Eldorado PH-200 photometer and power supply.

Results and Discussion

The diploid and tetraploid gametophytes are all recognizably characteristic of the species, quite similar in general appearance to the haploid. However, they are generally larger than the haploid, with considerable variation in size, especially among the tetraploids. The tetraploids are also inclined toward occasional aberrancies such as "ragged" margins and decreased lamellar development. Figure 2 shows habit photographs of representative prothalli from the three levels of ploidy, with corresponding metaphase figures of each.

After treatment with 10 ppm IAA for 48 hours, post-endomitotic figures were observed in prothalli of all three basic levels of ploidy. The chromosome levels in such nuclei were invariably one step above the basic ploidy, i.e., the haploids showed post-endomitotic diploidy, the diploids tetraploidy, and the tetraploids octoploidy. In *Osmunda cinnamomea* the normal mitotic chromosomes do not usually reveal chromatid separation until the earliest evidences of anaphase, when the centromeres separate (Figure 3*E*). Thus, the chromosomes up to metaphase appear single, unlike many other plants in which the double structure of the chromosome is clearly visible from prophase on to actual chromatid separation into chromosomes at the beginning of anaphase. In the post-endomitotic nuclei, however, the two sister chromosomes, each consisting of two chromatids, separate early in prophase, and only then are the typical diplochromosomes evident (Figure 3*B*). By prometaphase to metaphase the sister chromosomes have separated and are recognized only as pairs of identical chromosomes in close proximity (Figure 3*C*). This proximity is retained through metaphase and into early anaphase (Figure 3*D*), at which stage each sister chromosome can be seen to separate into two chromosomes, the previously indistinguishable chromatids.

The results of the photometric measurements of relative amounts of DNA in individual nuclei of untreated prothalli confirm these observations (Figure 4). The unduplicated levels of DNA were established from measurements on intact halves of anaphases and the duplicated levels from prophases. The C levels are shown at the bottom of the chart, and apply to all three graphs. In the haploid the expected range of DNA values would be from unduplicated to duplicated haploid (C



DNA CONTENT OF NUCLEI IN POLYPLOID SERIES OF FERN PROTHALLI

Figure 4

Photometric measurements of DNA (Feulgen) in individual nuclei of haploid, diploid, and tetraploid prothalli of *Osmunda cinnamomea*. The amounts of DNA in arbitrary units plotted against numbers of nuclei measured.

to 2C). That a second peak was found at the 2C level indicates that mitosis does not occur immediately upon completion of DNA synthesis. This delay between the processes of chromosomal replication and nuclear division is a necessary first step of endomitosis. If synthesis continues or resumes, without nuclear division, then the process of endomitosis is occurring. In the haploid this tendency was only slightly expressed. However, the basic conditions were evident, and some DNA values trailed off into the 2C to 4C range. In the diploid prothalli the basic levels would be from 2C to 4C. These were found, but with a more pronounced accumulation at the duplicated (4C) level, and a greater number of nuclei at the first endomitotic level, 4C to 8C. In the tetraploid prothalli the basic levels would be from 4C to 8C. Here most of the nuclei in this range were at the duplicated level. However, the great preponderance of nuclei was at the first level of endopolyploidy (8C to 16C).

Thus, it is apparent in this material that regardless of the initial level of ploidy, endomitosis occurs to produce nuclei of the same proportionate levels of endopolyploidy. It is interesting to note that the higher the basic level of ploidy the greater the tendency toward endomitosis, with respect to the numbers of cells entering into the process. However, considering the nuclear aspects of cell multiplication, this includes, in chronological order, chromosomal replication, nuclear division, and cellular division. These processes are separable. Endomitosis is evidence of the separation of chromosomal replication from nuclear division, just as the occurrence of binucleate cells is evidence of the separation of nuclear and cellular division. Very probably numerous factors can be limiting at any or all stages. That a process such as nuclear division, once initiated, tends to go to completion is suggestive of the possibility that there may be threshold levels which such limiting factors must attain before initiation of the process. It would be particularly interesting to know whether such requirements as, for example, an energy supply increase quantitatively and proportionately as the level

of ploidy, and if possible thresholds also increase. Differential responses of nuclei as a consequence of different levels of ploidy are known, the very technique of induced division of endopolyploids with higher levels of auxin being a relevant example. Very possibly a diploid nucleus also has a higher energy requirement for the synthesis of a chromosome set, as well as for the division process, than does a haploid. Then, if there be such a general factor which can be limiting both processes, there would be a problem of orderliness or priority. Obviously chromosomal synthesis must precede mitosis. However, if the former had a lower threshold, and all other factors being optimal but an energy supply being limiting, there would be the possibility of continued or resumed synthesis upon completion of one synthetic cycle. This type of speculative hypothesis would perhaps provide the simplest explanation for the occurrence of endomitosis on the basis of a single limiting factor. Conceivably the true situation can be much more complex. However, this simplified view does present a means of considering the problem of endomitosis which is at least not inconsistent with the available information. It lends itself readily to an interpretation of somatic polyploidy in differentiated cells of a plant system[10, 11]. It can also be applied to the present problem of the increased tendency toward endomitosis in individuals of higher basic ploidy. If such limiting factors are differentially operative and if the total economy of the higher ploid plants is not increased in proportion to the increased nuclear demands, then a continued expenditure of the available capital into the process of lesser initial investment in this tight economy could favor continued chromosomal replication, i.e., endomitosis. It is apparent, from such considerations as these, that further comparative quantitative physiological studies on a polyploid series of organisms such as the fern prothalli can provide information on the consequences of polyploidy which may be applicable to a better understanding of the significance of endomitosis.

Summary

An aposporously obtained polyploid series of gametophytes of the fern *Osmunda cinnamomea* was studied cytologically and cytophotometrically for the occurrence of endomitosis. Individuals of three basic levels of ploidy (1*n*, 2*n* and 4*n*) all showed endomitotic nuclei of the next higher level of ploidy, both in auxin-induced divisions and in microspectrophotometric measurements of DNA in individual nuclei of untreated plants. The photometric data indicated that an increasingly greater number of nuclei entered endomitosis in the diploid and tetraploid prothalli than in the haploid.

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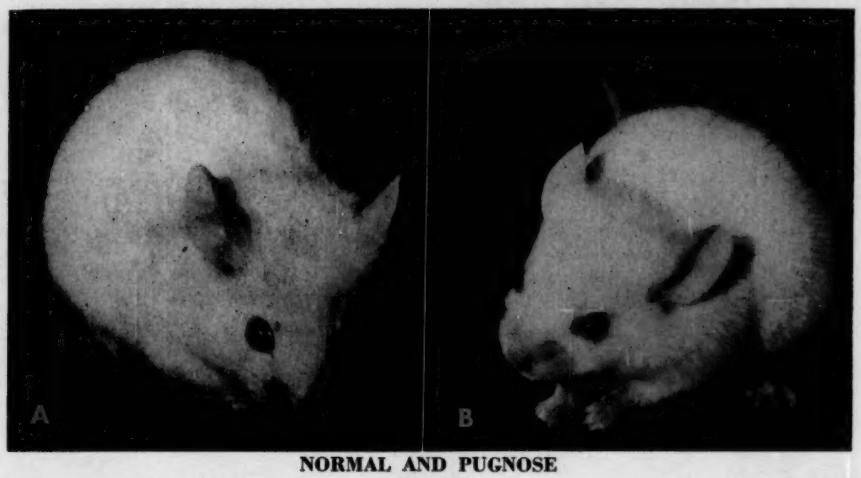
PUGNOSE—A RECESSIVE MUTATION IN LINKAGE GROUP 3 OF MICE

J. F. KIDWELL, J. W. GOWEN AND JANICE STADLER*



NORMAL AND PUGNOSE

Figure 5

A shows a normal S-strain mouse. *B* shows a pugnose (*pn*) of the S strain. Note reduced distance eyes to tip of nose, relatively broader forehead and somewhat Roman nose of pugnose as contrasted with normal (wild-type) head.

A RECESSIVE mutant, designated pugnose, symbol *pn*, arose *de novo* in 1934 in the S strain maintained by Gowen at the Rockefeller Institute for Medical Research. This paper presents a description of the mutant and relevant linkage data, as the gene is significant to radiation research.

Description

The gene expression is phenotypically distinguishable in the live mouse at 28 days. The most conspicuous feature, which suggested the name, is the relatively shorter, wider head. Greater width between the eyes and over the muzzle is also particularly noticeable. The forehead protrudes slightly, and there is a mild tendency towards a Roman nose. In more pronounced instances the skin over the nose is wrinkled. The eyes bulge slightly, as in thyroid-deficient animals. The lower jaw is wider than normal, but articulation is not affected. A further distinguishing characteristic of the live animal is a small knob at the posterior tip of the sternum. The skin seems to be too big for the pugnose mouse and, in consequence, wrinkles across the nose and ventral part of the body.

Adult pugnose mice average two to four grams lighter than their normal littermates, but there is much variation and some overlap into the normal weight range. Fertility is normal among the males, but is markedly reduced in the females. Many fail to conceive, and there is a high incidence of uterine prolapse at parturition. Many of the young die at birth, apparently as the result of birth injury. It is quite common for females to

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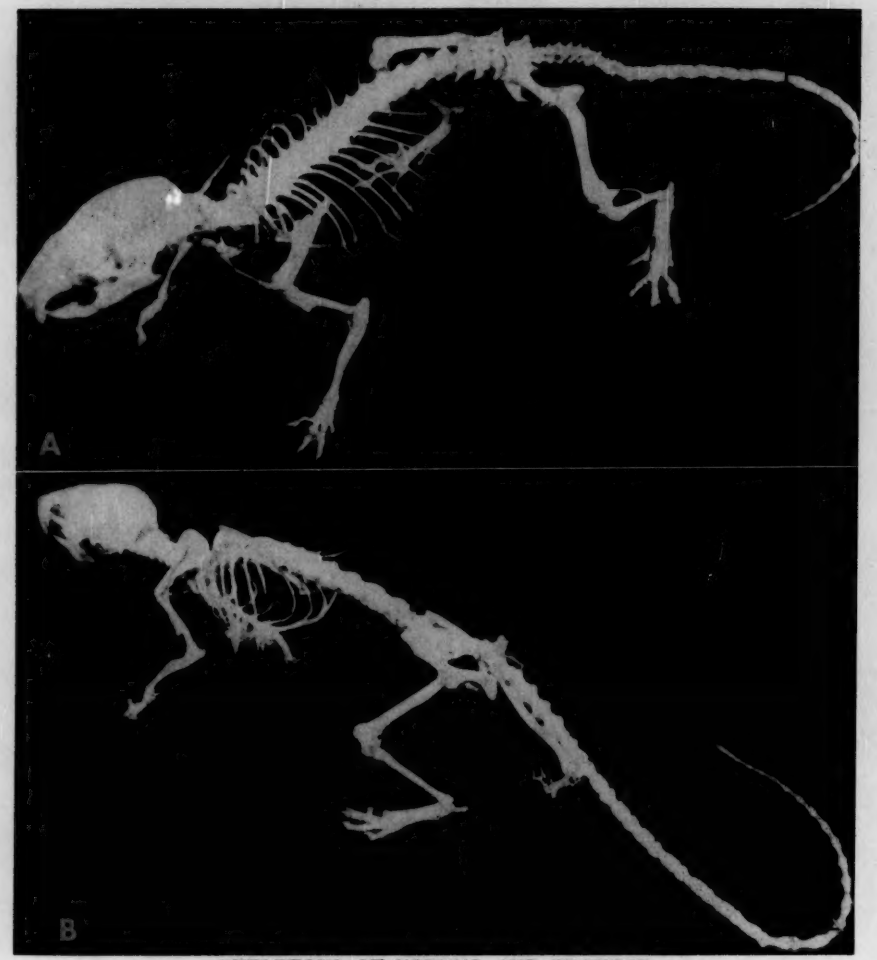

SKELETONS OF NORMAL AND PUGNOSE

Figure 6

Cleaned skeletons of normal S mouse (*A*) contrasted with pugnose S (*B*). The reduced frontal, parietal and nasal bones of the pugnose mutants are evident. Posterior tip of the sternum ends in a blunt knob and is bent ventrally.

be unable to deliver the young, and consequently to succumb. The number of embryos implanted is generally reduced. Mothering ability is often poor, and failure to lactate has been observed. An adult pugnose and a normal littermate are shown in Figure 5.

Examination of skeletons of cleaned, alizarin-stained pugnose and normal adults reveals that the parietal, frontal and nasal bones of the pugnose are relatively shorter and wider than normal, and slightly misshapen to produce the characteristic facial appearance. Also, the mandibles are relatively shorter and wider, and the incisors shorter and straighter than normal. The posterior tip of the sternum is bent ventrally. These features of the skeleton are shown in Figure 6.

There is a reduced survival to weaning among pugnose mice. In the absence of other mutants, 15 to 20 percent of the homozygous pugnose fail to reach 28 days of age. The exact stage or stages of loss has not been determined.

The phenotypic expression of pugnose resembles in some ways the effects of vitamin D deficiency. It is at least suggestive of an altered calcium metabolism.

Linkage Relations

The mutant behaves as an autosomal recessive. Repulsion F_2 and testcrosses indicate linkage with piebald in linkage group 3. Other repulsion F_2 tests with *c, p, se, si, a, bt, wa-2, b, v, ru, je, ln, fz,* and *f* produced sufficient cross-overs to indicate independence. Testcrosses with *Wa, Re, Mi^{wh}, Va* and *To* indicate no association. The symbols are those of Green and Dickie[4]. Relevant data for linkage group 3 are presented in Table I. A chi-square analysis of the F_2 data indicates linkage between pugnose and piebald, a 3:1 segregation for piebald but a disturbed segregation for pugnose. Many methods have been developed to estimate linkage intensity from F_2 data[5,6]. The "product method" developed by Fisher[1] and by Fisher and Balmukand[3] provides an unbiased and efficient estimate when the disturbed segregation is due to reduced viability of one of the mutant classes. The percent of recombination is estimated from the F_2 data by this method as 27.9 ± 3.0.

The testcross results (heterozygous males) are not in close agreement with the F_2 data. Chi-square analysis indicates no deviation from a 1:1 ratio for either piebald or pugnose. There is, however, an obvious deficiency in the piebald pugnose class derived from heterozygous females. It is possible that there is also a deficiency of piebald pugnose mice in the F_2 progeny. Such a deficiency would not noticeably disturb the 3:1 segregation for piebald because of the small expected numbers of piebald pugnose individuals. There is no obvious explanation for the difference in viability of pugnose mice in the F_2 and the testcross progenies.

An exact method of estimating percent of recombination from testcross data when ratios are disturbed has been developed by Fisher[2], but it requires both coupling and repulsion data. Since coupling data are not available, an exact estimate is not possible. An estimate which is only slightly biased can be obtained by taking account of the deficiency. The estimate is not fully efficient or sufficient but is the best obtainable. These data yield an estimate of 35.9 ± 2.2 percent recombination between piebald and pugnose.

Testcross data for heterozygous males is limited due to the low productivity of pugnose females. It suggests the same rate of recombination in males and females.

None of the estimates are entirely satisfactory. If there is a deficiency of piebald pugnose mice in the F_2 progeny, this estimate is also biased since the product method accounts for disturbance

TABLE I. Results of linkage tests of pugnose and piebald and macrocytic anemia

Mating		Progeny				
♂	♀	+ *pn*	*s* +	*s pn*	+ +	Total
$\frac{s\ +}{+\ pn}$	$\frac{s\ +}{+\ pn}$	191	220	16	501	928
$\frac{s\ pn}{s\ pn}$	$\frac{+\ pn}{s\ +}$	154	153	46	86	439
$\frac{+\ pn}{s\ +}$	$\frac{s\ pn}{s\ pn}$	19	12	3	7	41
		+ *pn*	W^a +	W^a *pn*	+ +	
$\frac{+\ pn}{+\ pn}$	$\frac{+\ pn}{W^a\ +}$	45	70	42	60	217
$\frac{+\ pn}{W^a\ +}$	$\frac{+\ pn}{+\ pn}$	10	9	12	8	39

in a single factor only. Three-point tests with piebald and hairless are being made. They will provide a more accurate location of the gene but will require some time to complete.

The data indicate no association between pugnose and macrocytic anemia. From this, it follows that the gene order is *W*^{e} *s-pn*. This indicates that *s* is not terminal or subterminal, and that linkage group 3 is much larger than previously observed. Russell[7] observed a high X-ray-induced mutation rate at the *S* locus and suggested that it might, at least in part, be due to terminal deficiencies. This could be the case, but the deficiencies would be fairly large. This hypothesis may be tested by including the pugnose gene in such tests.

Summary

A mutation in the mouse called pugnose, symbol *pn*, is described. It is located in linkage group 3, and apparently toward the terminal end of the chromosome away from piebald. Twenty-eight to 36 percent recombination with piebald, depending on the type of mating, was observed in these experiments.

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A MUTANT LOCUS DETERMINING ABNORMAL MORPHOLOGY AND ASCOSPORE LETHALITY IN NEUROSPORA

JAY C. MURRAY AND ADRIAN M. SRB*

MUTATION in *Neurospora crassa* is capable of giving rise to bizarre deviations from the morphology characteristic of the wild-type[11]. Morphological mutants of this kind appear to constitute favorable material for studies of genetics and development. The present paper is concerned with two independently derived mutant strains that are characterized by similar deviant morphology and by the fact that the genes determining this morphology behave as ascospore lethals. When either of the morphological mutant strains is crossed to wild-type, the resultant asci contain eight black spores, as is normally the case for *N. crassa*. However, only two of the four spore pairs in each ascus are capable of germination. The spores that germinate always give rise to wild-type cultures. Morphologically, the viable spores are indistinguishable from those incapable of germination. The fact that inability to germinate shows a simple segregational pattern in the ascus designates the presence of a lethal gene. Although the two independently derived mutant genes exist only in cultures of the same mating type and therefore cannot be put into the same cross, their linkage relations, their similar morphological effects, and their common effect of lethality justify the conclusion that these genes are allelic. Because of the dual effects on growth habit and ascospore viability, the mutant locus is designated *col-le;* and the two mutant stocks are called *col-le-1* and *col-le-2* in this paper.

Materials and Methods

Mutant *col-le-2* was isolated from wild-type strain 74A of *Neurospora crassa* after treatment of the conidia with beta-propiolactone. The method of isolation was essentially as described by Woodward *et al.*[13] As pointed out by Srb[11], this method was designed primarily for the isolation of biochemical mutants; but morphological mutants able to grow on minimal medium are also recovered in relatively high frequency. *Col-le-1* was also isolated from wild-type strain 74A by similar methods, but after the conidia had been treated with acriflavin. Since no other mutants were isolated from the acriflavin-treated material, *col-le-1* may well have arisen spontaneously.

Strain 77a, the opposite mating type homologue of 74A, was the wild-type strain utilized in the crossing experiments. The temperature-sensitive colonial, *cot,* used as the genetic marker for establishing linkage relationships was obtained from Mary B. Mitchell of the California Institute of Technology.

The culture media, unless otherwise stated, were those described by Beadle and Tatum[2]. Colonies to be used for study of their morphology were grown in petri dishes containing 30 ml. of minimal medium solidified with 2.5 percent agar. Because of their compact aerial mycelium, colonies of *col-le-1* and *col-le-2* could only be examined microscopically in cross section. The sectioning was carried out by freezing the colonies and the blocks of agar on which they grew with carbon dioxide gas, and then hand sectioning the frozen material. For the study of hyphae, cultures were grown on agar blocks which had been placed on microscope slides and covered with a cover slip. The cover slip forces the mutants to form a thin mycelium in

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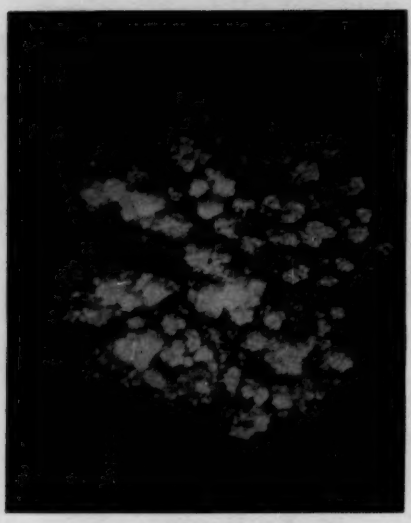


GRANULATED MYCELIUM
Figure 7

Colony of *col-le* on an agar surface after six days growth. 8×.

and along the agar surface where they can be examined without further preparation.

Since these mutants are ascospore lethals and also aconidial, cultures can only be maintained by transfer of chunks of mycelium. Chunks of mycelium were also used in making the crosses involving the mutants.

Genetic data were obtained both from ascospores isolated at random and from ascospores isolated in order from asci.

The centromere distances obtained for the mutant genes were corrected for double crossovers by the use of the graphs presented by Barratt *et al.*[4]

Observations and Results

Description of morphology

As distinguished from wild-type *Neurospora crassa,* both *col-le-1* and *col-le-2* are characterized by a restricted colonial growth habit and by a highly compact aerial mycelium. When the colonies are very young, their surfaces appear to be rather smooth. However, by the time cultures are 48 hours old, the compact aerial mycelia have developed a granulated appearance (Figure 7). The hyphae branch much more frequently than those of wild-type and many of the branches remain very short (compare Figure 8*A* and *B*).

Studies of the development of characteristic mutant colonies show that if a few hyphae are made to lie flat on an agar surface, many branches arise from them and grow aerially (Figure 8*C*). These aerial hyphae branch very frequently (Figure 8*D*) and form a compact aerial mycelium (Figure 8*E*). Cross sections of the colonies show that many hyphae also grow into the agar medium, forming a submerged mycelium. The submerged mycelium is more diffuse than the aerial mycelium; but the hyphae have numerous short branches (Figure 8*E*). The aerial and submerged hyphae formed while the colonies are young are slightly constricted (Figure 8*A*); but those formed in older colonies become swollen and highly articulated, the numerous short branches having the appearance of large, sub-globose buds (Figure 8*F*).

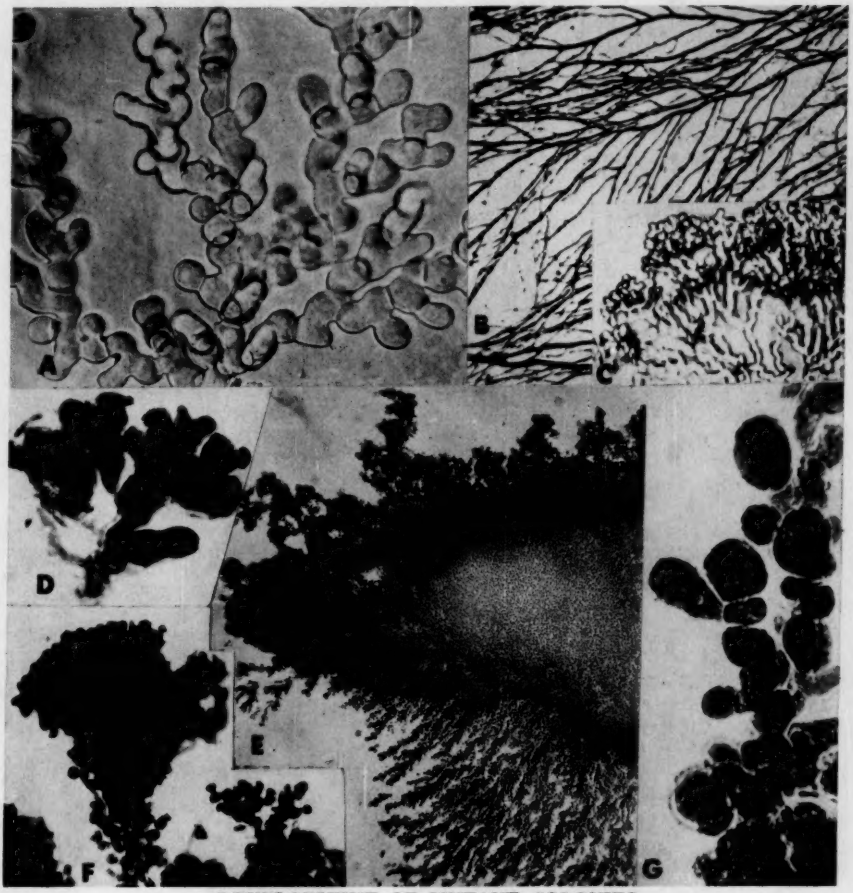
The granulated texture of the older colonies is produced when isolated hyphae grow out of and above the compact aerial mycelium, where they form clusters of branches which protrude above the compact mycelium (Figure 8*E* and *G*).

Genetic studies

Although the morphological mutants are not recovered among the ascospore progeny from crosses between the mutants and wild-type, the distribution in the asci of viable and non-viable spore pairs shows that these mutants are due to a mutation which behaves as a single

TABLE I. Frequencies of parental and non-parental ascospore segregants from crosses involving *col-le* with an independent marker (*Aa*) and with a linked marker (*cot*)

Marker	*col-le* parent	Progeny	
		parental	non-parental
A, a	–1	97 (*a*)	96 (*A*)
A, a	–2	43 (*a*)	53 (*A*)
cot	–1	537 (*cot*)	74 (wild type)
cot	–2	385 (*cot*)	70 (wild type)



DEVELOPMENT OF MUTANT COLONIES

Figure 8

A—Hyphae of *col-le* growing under a cover slip. 500×. *B*—Hyphae of wild-type growing on an agar surface. 50×. *C*—Mycelium of *col-le* growing on an agar block four hours after the cover slip was removed, showing numerous aerial hyphae beginning to grow up from the hyphae. 150×. *D*—An aerial hypha of *col-le* dissected from a colony four hours after the cover slip had been removed from the agar block. 500×. *E*—Vertical section through a colony of *col-le* showing the compact aerial mycelium, the diffuse submerged mycelium, and the outbursts of aerial hyphal growth which form the granulated texture of the aerial mycelium. 50×. *G*—Swollen and articulated hyphae from a colony six days old. *F*—A cluster of hyphae which protrude from the compact aerial mycelium and give the granulated texture to the aerial mycelium. 200×.

gene. When a second genetic marker is introduced into crosses involving *col-le*, only two of the expected four genetic classes occur among the resulting ascospore progeny (one parental and one non-parental class). If the two classes occur in a 1:1 ratio, *col-le* and the other marker can be presumed to be genetically independent. On the other hand, if the parental class is preponderant, then linkage is suggested. The data presented in Table I, derived from ascospores isolated at random, suggest that *col-le-1* and *col-le-2* are independent of the mating type

locus (*Aa*) and are linked to *cot*, which is reported to be located on the right arm of chromosome 4 [7].

A test for homogeneity of the above recombination frequencies of *col-le-1* indicates the data from the two crosses are homogeneous. From these data and from the centromere distances presented in Table II, *col-le-1* and *col-le-2* can be assumed to be allelic, at least in the classical sense; the locus, therefore, is referred to as *col-le*. The data presented in Table I show that *col-le* is located approximately 13.5 map units from *cot*. Since the data presented in Table II indicate centromere distances of 17.3 map units for *cot* and 26.2 map units for *col-le*, *col-le* must be in the distal to *cot*.

The same gene order can also be established from the data for ascus segregation presented in Table III. If two genes are located in the same arm of a chromosome, a preponderance of the second division segregations of the proximal locus should be associated with second division segregations for the distal locus. As shown in Table III, the second division segregations for the two loci are highly associated, indicating that they exist in the same arm of chromosome 4.

The order in which the two genes are arranged in the chromosome can be established from the frequency of the ascus segregation patterns presented in Table III. If *cot* were distal to *col-le*, then type 3 should be the result of a two-strand double crossover and type 4 of both single-strand and three-strand double crossovers. With this arrangement, type 4 would occur more frequently than type 3. On the other hand, if *col-le* were distal, then type 3 would result from both single and three-strand double crossovers. Since the data presented in Table III show that type 3 occurs more frequently than type 4, *col-le* must be located distally to *cot*.

Discussion

In addition to the colonial lethal mutants described here, other lethals in *Neurospora* have been previously described[2,6,10,12]. Dodge[3] isolated a mutant that has almost normal mycelium but gives rise, in asci homozygous for the mutant gene, to spores that abort early in development. The mutant described by Stadler, called *asco*, gives wild-type vegetative growth in the presence of an exogenous supply of lysine. However, when *asco* is crossed, the asci almost invariably contain four black spores, which germinate normally to form wild-type cultures, and four light-colored spores which fail to germinate. The mutant described by Lindegren and Lindegren[6] has an aberrant mycelium. After crosses between this mutant and wild-type, the resultant asci contain eight normal appearing spores, all of which are capable of germination. But mycelia derived from four of the spores die shortly after germination. The mutant studied by Sheng[10], called "natural death" (*nd*), grows normally for some time after the spores germinate; but following this period, progressive cessation of growth culminates in death. The *nd* gene can only be maintained in heterocaryons. Considered with reference to these other mutants in *Neurospora*, *col-le* represents a new member in a series of genes that give rise to lethality at various stages in the life cycle and that have their various detectable effects on the physiology or development of the organism.

In certain organisms that have been subjected to long and intensive genetic study, for example *Drosophila*, series of lethal genes have been known for some time and have been

TABLE III. Ascus segregation in crosses between *cot* and *col-le-1*

Type of segregation	Segregation				No. of asci
1	*le*	*le*	*cot*	*cot*	49
2	*le*	*cot*	*cot*	*le*	24
3	*le*	wild	*cot*	*le*	15
4	*le*	*le*	*cot*	wild	5
5	*le*	*le*	wild	wild	1
					94

TABLE II. Centromere distances for *col-le-1* and *col-le-2*

Strain	Number of asci 1st div. seg'n	2nd div. seg'n	Corrected map distance from centromere value	range
col-le-1	61	48	23.8	19.2-34.8
col-le-2	25	33	32.2	22.0-43.5
total *-1* and *-2*	86	81	26.2	24.0-31.7
cot	90	43	17.3	13.0-24.5

useful in studies of development[5]. More recently, Esser and Straub[4] have reported gene mutations in the fungus *Sordaria macrospora* which block the development of the perfect fruiting structure at different stages of development. The data discussed here, together with the data presented by Esser and Straub, suggest that development in fungi can be partitioned on the basis of the action of lethal genes and that a study of lethals may lead to increased understanding of developmental phenomena in fungi, as has been the case in insects and mammals. The effects of the *col-le* locus on the habit and structure of hyphae, with their consequences for colonial form, offer a point of attack for such studies.

At present, the *col-le* mutants may be thought of as representing an instance of pleiotropy, in that a genetically identifiable spot in a chromosome that behaves as a single locus governs both lethality and a drastic alteration of morphology. However, these aberrancies may both stem from some primary physiological lesion, or, alternatively, the two effects may be the result of a deficiency of two or more genes in the mutant strains. The fact that neither *col-le-1* nor *col-le-2* has been observed to back-mutate is at least consistent with the latter hypothesis. Further analysis is required for resolution of the alternatives.

Summary

In *Neurospora crassa*, a locus designated *col-le* determines ascospore lethality and extreme morphological deviation from the wild-type. The *col-le* locus is about 26 map units from the centromere of its chromosome and is linked to *cot*, therefore placing *col-le* in linkage group 4. Two independently derived and phenotypically similar *col-le* mutants have been found. The mutant genes appear to be allelic. The *col-le* mutants, as maintained in vegetative culture, have a restricted colonial growth habit and a highly compact aerial mycelium. Morphological analysis shows that the hyphae of *col-le* differ structurally from those of wild-type, and that the aerial hyphae of the mutants branch more frequently. Ascospores having the *col-le* gene are morphologically normal but unable to germinate.

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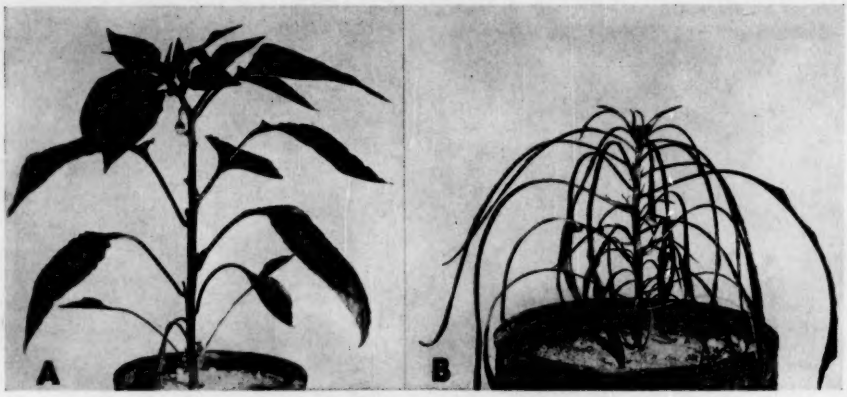
INHERITANCE OF MUTANT-1 PHENOTYPE IN THE PEPPER

A. A. Cook*



NORMAL AND MUTANT PHENOTYPES

Figure 9

Phenotypes noted in (No. 471) F_3 progeny. *A*—Normal (ca. 0.5×). *B*—Mutant-1 (ca. 0.3×).

While investigating the nature of inheritance of a specific reaction in pepper (*Capsicum annuum* L.) to tobacco mosaic virus, a mutant phenotype was observed in an F_3 progeny of a cross between S.C. 46252 and Yolo Wonder. The extent of variation in this mutant type from the normal-appearing sib plants was so extreme as to make it hardly recognizable as a pepper. The following is a report of an attempt to determine the heritability of the characters comprised in this phenotype, herein referred to as mutant-1.

Description of Mutant Plants

The leaves of the normal-appearing plants in these studies (Figure 9*A*) were ovate with acute, almost acuminate tips, pinnate venation, and entire margins. Veins were prominent on the lower side of the lamina, the base of which was rounded and slightly decurrent. The petiole was slender and usually half or more the length of the lamina. Leaf and petiole frequently measured somewhat more than twice as long as the widest portion of the lamina. Lobes of the truncate, synsepalous, short tubular calyx were represented by short teeth (Figure 10*A*). The rotate corolla consisted of a short tube and five to seven lobes that measured about half the length of the corolla (Figure 10*C*). Epipetalous stamens were connivent with the extrorse anthers borne on filaments narrowed upward. The stamens approximately equaled the stigma. The superior, globular ovary, consisting of two to four carpels, was not depressed. The single style was erect, straight, cylindrical and stout with a blunt, slightly enlarged stigma.

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FLOWERS FROM NORMAL AND MUTANT PLANTS

Figure 10

A—Longitudinal section of normal flower. *B*—Longitudinal section of mutant-1 flower. *C*—Face view of normal flower. *D*—Face view of mutant-1 flower (ca. 10×).

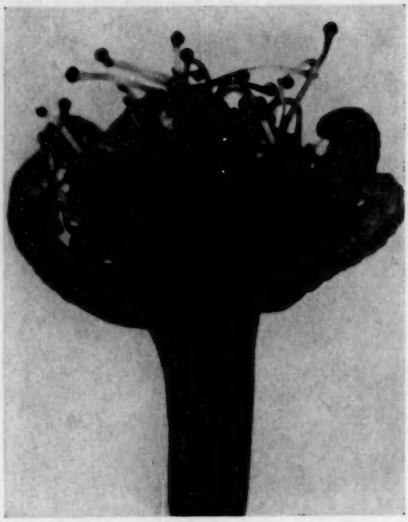
Plants of the mutant-1 phenotype (Figure 9*B*) were characterized by noticeably more narrow cotyledons and a slower initial growth rate than normal-appearing plants, but they eventually reached the approximate size (height) of normal plants. Internodes of mutant-1 plants were often shorter than usual. The leaves drooped and were narrowly linear-lanceolate with acuminate tips and undulate, irregular and usually thickened margins. Venation in the laminae was indistinctly pinnate and the leaf bases decurrent and barely distinguishable from the petioles. Petioles had indistinctly thickened margins and were channeled almost to the base. Although the leaves measured six inches or more in length (including petioles), the laminae were up to one-half inch at the widest portions and usually much less. Sepals were represented by distinct, fleshy, finger-like projections with distinctly incurved tips but were barely, if at all, connate (Figure 10*B*). The corolla was rotate and composed of nar-

row, strap-shaped, channeled and almost involute lobes with irregular margins (Figure 10*D*). The petals were also barely connate at the base. Filaments of the epipetalous stamens were narrowed upward, irregularly oriented and bore extrorse anthers. The ovary wall was represented by four separate, broadly ovate segments connate at the base and depressed at the tips where there was borne on each segment an aborted style-like structure with apparent stigma. The placental tissue was enlarged and irregular with the ovules represented by aborted ovule-like structures borne on apparently elongated funiculi bent in various degrees of orientation (Figure 11).

Inheritance

To determine if the mutant-1 condition was associated with presence of a virus, scions from pepper plants of the virus-indicator variety (Tabasco *C. frutescens* L.) were grafted onto the three (one was lost in transplanting) original mutant-1 plants. No symptoms indicative of virus infection developed in any of the Tabasco scions, which grew quite well on the mutant-1 stocks.

All mutant-1 plants were female-sterile, irrespective of pollen used in attempts at fertilization. Pollen from mutant-1 plants was fertile, and as a consequence, it was possible to obtain



MUTANT-1 FLOWER DISSECTED
Figure 11

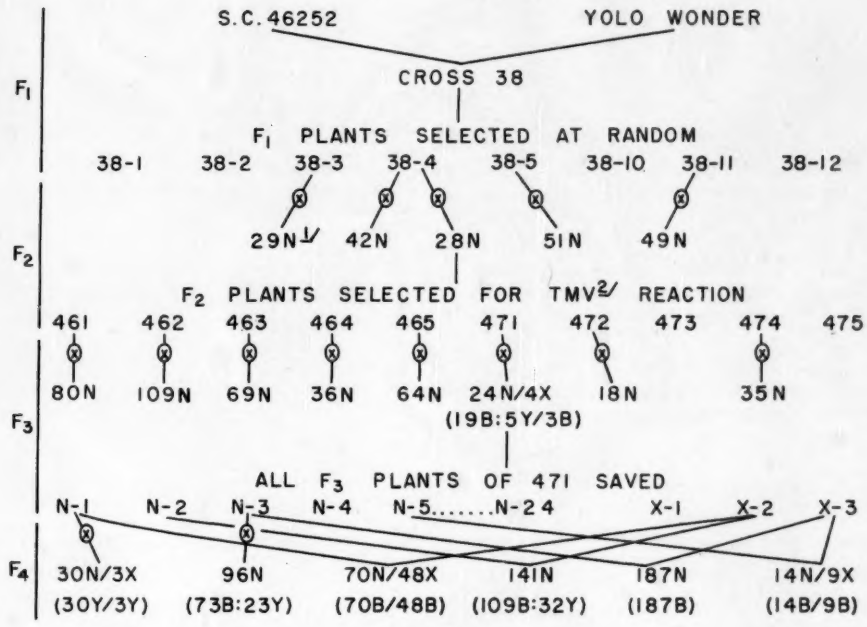
Mutant-1 flower witn corolla and ovary wall segments removed (ca. 10×).

crosses with plants of unrelated pedigree as well as sib-crosses using normal-appearing plants of the No. 471 progeny (Figure 12) as female parents. At least one sib-cross onto each normal plant of this (No. 471) progeny was attempted using pollen from a mutant-1 plant selected at random. Also, in so far as

TABLE I. Segregation and chi-square analysis according to phenotype and anther color in progenies from normal-appearing sib plants selfed

Number of plants				Assumed seg. ratio				
Normal		Mutant		Pheno-	Anther		P values‡	
Blue anthers	Yellow anthers	Blue anthers	Yellow anthers	type (N:X)*	color (B:Y)†	T	P	I
35				1:0		.99	.99	–
					1:0	.99	.99	–
259	89			1:0		.99	.99	.99
					3:1	.5–.7	.8–.9	.3–.5
251		77		3:1		.2–.3	.5–.7	.1–.2
					1:0	.99	.99	.99
	84		21	3:1		.05–.10	.2–.3	.05–.10
					0:1	.99	.99	.99
34	18	12	3	3:1		.3–.5	.5–.7	–
					3:1	.05–.10	.2–.3	–

*N—normal phenotype; X — mutant-1 phenotype.
†B—blue anthers; Y — yellow anthers.
‡Total, pooled, and interaction χ^2 values, respectively.



PEDIGREE OF MUTANT-1

Figure 12

Pedigree and appearance of plants in some progenies related to (and including) the progeny in which mutant-1 plants were first observed. (N — normal appearing plants; X — mutant-1 plants; B — blue anthers; Y — yellow anthers; TMV — tobacco mosaic virus.)

possible, one or more selfed fruits were harvested from each normal plant. Five of the normal plants had yellow anthers while the anthers of the other 19 normal plants were blue, as were the anthers of the three mutant-1 plants. Some typical results obtained from the progenies described above are presented in Table I.

The mutant-1 phenotype, with all characters occurring *en bloc,* was found to occur according to the expected frequency of a single recessive gene in the homozygous condition, and did not appear in progenies from crosses with plants of pedigree unrelated to the No. 471 progeny. Anther color (blue vs. yellow) was inherited independently of the mutant-1 phenotype and was also determined by a single allelic pair, the double recessive being yellow. The probable genotypes of the plants in No. 471 progeny, as determined from segregation in the selfed progenies, were as follows (assuming *B/b* for anther color and *M/m* for mutant-1 genotype):

BBMM — 1 *BBMm* — 4 *BBmm* — 2
BbMM — 5 *BbMm* — 2 *Bbmm* — 1
bbMM — 0 *bbMm* — 3 *bbmm* — 0

Undetermined—nine plants.

Segregation for both the mutant-1 phenotype and anther color in the sib-cross progenies, as illustrated in part in Figure 12, provided supporting evidence that the above genotypes of the normal and mutant-1 plants were correct. From these sib-cross progenies, it was possible to derive probable genotypes for three other plants in the (No. 471) progeny, *viz, BBMM*—one plant and *bbMm*—two plants. Thus a probable genotype was obtained for 18 of the 24

normal and all three of the mutant-1 plants in the original (No. 471) progeny.

Discussion

Although there have been reported instances of a spontaneous tetraploid[7] and polyembryony[10] in pepper, no mutant type comparable to the one described herein has been reported. Mutant phenotypes have been reported in lupine[3], pea[8], *Solanum capsicastrum*[1], broad bean[6], and corn[11]. Evolutionary significance was suggested for the latter. It is doubtful that the mutant type herein considered represents an example of "systemic mutation" as defined by Goldschmidt[4]. Such an extreme phenotype could result from "dichotomic pleiotropy"[5], although tests such as those reported by Mathan *et al.*[9] were not conducted.

The inheritance of blue vs. yellow anther color and the observation that different shades of blue anther coloring occur were in accord with the results of Deshpande[2].

Attempts to associate the mutant-1 phenotype in pepper with a virus infection were comparable, though less extensive, to the negative results of Edwardson and Corbett[3] who studied the inheritance of a similar condition in lupine.

Summary

A female-sterile mutant, referred to as mutant-1 and discovered in an F_2 progeny from a single selfed F_2 pepper plant selected for a specific reaction to tobacco mosaic virus, is described. As determined from out-crossed, sib-crossed, and progenies from selfed normal-appearing sib plants, the aberrant characters exhibited by all mutant-1 plants occurred *en bloc* and according to the expected frequency of the double recessive of a single allelic pair. Anther color was inherited monofactorially and independently of the mutant-1 phenotype with blue dominant to yellow.

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LINKAGE OF WABBLER-LETHAL AND HAIRLESS IN THE MOUSE

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THE mutation, wabbler-lethal, occurred in the pirouette stock of mice at this laboratory in 1948. It has been described in detail by Dickie *et al.*[1] Since the publication of that paper, in which wabbler-lethal is described as an incompletely dominant gene and the symbol *Wl* is suggested, additional breeding tests have shown the heterozygous animal to be distinguishable from the normal only in exceptional cases. Therefore, this gene is more conveniently treated as a recessive, and it is proposed that *wl* be assigned as the proper symbol for this mutation.

The results of several linkage tests which show that wabbler-lethal is located close to hairless (*hr*) in linkage group 3 are presented in this paper.

Results

The autosomal recessive gene hairless (*hr*) was present in the pirouette stock in which *wl* arose. A total absence of the double recessive, *hrhrwlwl*, was noticed among the progeny of crosses segregating for both *hr* and *wl*. This suggested the possibility of a close linkage between the two genes. In order to determine the location of *wl* in relation to *hr*, linkage test matings were set up. The data from all crosses are given in Table I.

Over 1700 offspring were classified in a repulsion intercross (cross 1) and no *hrhrwlwl* mouse was recognized. To determine whether any crossing-over had occurred, 25 hairless males from the progeny of this cross were tested for single cross-overs by matings with known heterozygous females, *hr+/+wl* (cross 3). A minimum of 12 offspring were raised and classified from each test mating. Of the 25 hairless males tested four were found to carry *wl*. The recombination proportion, p, and its variance, V_p, can be estimated from these data by the formulas:

$$p = \frac{a}{2N-a}$$

and

$$V_p = \frac{p(1-p)(1+p)^2}{2N}$$

where a is the number of mice found to be carrying *wl*, and N is the number

TABLE I. Linkage tests with wabbler-lethal

Cross	Genotype of parents ♀ × ♂	Phenotype of progeny ++	*hr+*	*+wl*	*hrwl*	Total	Recombination % ± S.E.	χ^2*wl**
1	*hr+/+wl* × *hr+/+wl*	844	436	426	0	1706		0.001
2	*hrwl/++* × *hrwl/++*	399	10	10	132	551	3.66 ± 0.74	0.175
		hr+/hr+		*hrwl/hr+*				
3	*hr+/+wl* × *hr+/+wl*	21		4		25	8.70 ± 4.60	
Crosses 2 and 3 combined							3.79 ± 0.72	
		*W*v+*	*++*	*W*vwl*	*+wl*			
4	*W*v+/+wl* × *++/+wl*	102	140	22	53	317	40.70 ± 4.79	0.304
5	*++/+wl* × *W*v+/+wl*	41	39	14	14	108	49.38 ± 8.21	0.148
Crosses 4 and 5 combined		143	179	36	67	425	42.89 ± 4.13	0.132

*χ^2 for segregation of *wl*.

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This work was supported in part by research grants NSF G-6200 from the National Science Foundation and ACS E-162 from the American Cancer Society. The authors are indebted to Dr. Margaret C. Green for the linkage formula for cross 3 used in this paper.

tested. The recombination thus estimated is 8.70 ± 4.6 percent.

From the hairless mice carrying *wl* the coupling chromosome was propagated to produce a coupling intercross. Data from this cross (cross 2) which produced 551 offspring gave a recombination estimate (calculated with the aid of Finney's tables[2]) of 3.66 ± 0.74 percent. The combined estimate from the data of crosses 2 and 3, obtained by weighting each estimate by the reciprocal of its variance, is 3.79 ± 0.72 percent.

The linkage tests between viable dominant spotting (W^v) and *wl* (crosses 4 and 5) gave a recombination of 42.89 ± 4.13 percent. This estimate may not be reliable because of the abnormal segregation of W^v in cross 4, the reason for which is not known. The estimate is nearly identical with the recombination of 42.10 ± 1.30 percent found by Gates and Pullig[3] between dominant spotting (*W*) and hairless, and does not allow any conclusion concerning the order of *W*, *hr*, and *wl*. Wabbler-lethal segregated normally in all crosses.

Summary

Wabbler-lethal is very closely linked to hairless in linkage group 3. The recombination is 3.79 ± 0.72 percent.

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✦

A note on interchromosomal effects on crossing-over induced by reversed acrocentric compound X-chromosomes in *Drosophila melanogaster*

Pierre Hart and L. Sandler*

Interchromosomal effects are conventionally studied by making a chromosome pair structurally heterozygous—usually for one or more inversions—and measuring recombination in some other chromosome pair. Generally, as the structural heterozygosity is increased, crossing-over in the non-homologous chromosome pair increases[8, 9]. One of the complications in experiments of this sort is the difficulty of resolving the separate effects of the two factors that are invariably introduced simultaneously into the experiment: (1) the effect of rendering portions of the genome structurally heterozygous as opposed to, (2) the effect of the altered exchange values in the structurally heterozygous pair produced by the structural heterozygosity.

It was considered possible that some resolution of this problem might be attained through the use of the compound X-chromosome known as the reversed acrocentric[4]. This chromosome is composed of two euchromatically complete X-chromosomes attached in reverse sequence to a subterminal centromere. The chromosome thus synapses by folding back on itself. This chromosome has the special property whereby XX females have a very different distribution of the different rank exchanges than XXY females[5, 6]. Consequently, in this system, exchange can be altered without the addition or subtraction of inversions.

The results of measuring crossing-over in the third chromosome in females carrying reversed acrocentrics (with and without a Y-chromosome) or else corresponding free-X combinations suggests that the frequency distribution of tetrads by rank is at most a minor component of the interchromosomal effect on crossing-over.

Materials, Methods and Results

Reversed acrocentrics are produced by a crossover between the distal heterochromatic

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region of *In(1)sc*^{8} (a long inversion of the X-chromosome including most of the proximal heterochromatin) and the base of a normal chromosome so paired that the resulting compound has but one centromere[4]. The component chromosomes of the compound synapse with themselves and cross over. Although only exchanges of rank 2 or more are detectable, it is possible to do a tetrad analysis and determine the relative frequencies of the various rank tetrads. For reversed acrocentrics without a homolog, most of the tetrads (about 70 percent) have no exchanges, a very few, if any, have one exchange, and the remainder are of rank 2. In reversed acrocentric-bearing females also carrying *FR2* (equivalent to the long arm of the Y-chromosome and carrying the distal uninverted region of *In(1)sc*^{8} including *y*+) exchange is increased such that typically E_0 equals 0.44, E_1 equals 0.12 and E_2 equals 0.44 (E_i is the frequency of exchange of rank i). Heterochromatic duplications for X-chromosome heterochromatin also act as homologs for the compound, but do not affect exchange. The evidence for the conclusions just presented is found in the analyses of Sandler[5, 6]. Structurally very similar to reversed acrocentrics, *sc*^{8} heterozygotes most probably show about 50 percent single and 50 percent double (or higher rank) exchange tetrads[2, 10]. Thus, comparing the interchromosomal effects produced by *sc*^{8} heterozygotes and reversed acrocentrics with and without *FR2* allows a consideration of a considerable variation in exchange distribution with, however, the same inversion heterozygosity.

The experiments performed were as follows. From a reversed acrocentric line heterozygous for *In(1)AB* (a fairly small, medially placed inversion and inseparable from the normal allele of *v*) and *v*, non-crossover reversed acrocentrics still heterozygous for *In(1)AB* and *v* and also reversed acrocentrics homozygous for *v* (from double exchanges which cause homozygosis for chromosome regions, as in attached X-chromosomes) and therefore lacking *In(1)AB* were recovered. These were all made heterozygous for the third chromosome dominants, *Gl Sb* (following the results obtained by Schultz and Redfield[7]). Some were so mated that they carried either *FR2* or *Dp135* (an X-chromosome duplication that acts as a homolog for the compound and is marked by *y*^{2}) and then mated to YSX.YL, *y B*/*o* males. These males carry an attached XY-chromosome containing the mutants *y* and *B* and they have no free Y-chromosome. Crossing-over between *Gl* and *Sb* was measured. The results are given in Table I.

It can be seen that there is a striking interchromosomal effect, with crossing-over in the *Gl-Sb* region being highest in the *sc*^{8}/*AB* combinations, next highest in the *sc*^{8}/+ combinations and lowest in the sequentially normal controls. However, the various sets sharing the same inversion combination are fairly uniform. Since, as argued above, the various sets that share the *sc*^{8}/+ inversion combination differ among themselves widely in the exchange distribution in the X's, it may be concluded that such changes have relatively little effect on interchromosomal effects on crossing-over.

Discussion

While the crossover frequency in the *Gl-Sb* region calculated for the +/+ stock in Table I is low relative to the standard value (16.8), it agrees very well with the value obtained by Schultz and Redfield[7] in other work pertaining to the interchromosomal effect.

Although there is no doubt that the various inversion combinations form groups each distinct from the other, these groups are heterogeneous by usual statistical criteria. There is, in fact, the particular relation that the

TABLE I. Recombination between the markers *Gl* and *Sb*, measured in females of a number of different sex chromosome constitutions. (Symbols are defined in text.)

Inversion type	Sex chromosome constitution of parental female	Phenotype of progeny				Percent recombination
		Gl Sb	*Gl* +	+ *Sb*	+ +	
Normal	+/+	4015	454	488	3762	10.8
sc^{8}	*sc*^{8}/+	1209	236	242	1252	16.3
	*R.A.**/*O*	981	235	254	1158	18.6
	R.A./FR2	1628	281	336	1759	15.4
	R.A./Dp135	1640	351	363	1726	17.5
sc^{8}*AB*	*sc*^{8}	2154	754	821	2482	25.4
	R.A.(AB)†/*O*	1044	390	468	1307	26.7
	R.A.(AB)/FR2	766	242	269	986	22.6
	R.A.(AB)/Dp135	1381	381	416	1492	21.7

*Reversed acrocentric compound not heterozygous for *In(1)AB*.
†Reversed acrocentric compound heterozygous for *In(1)AB*.

reversed acrocentric (either with or without the *AB* inversion) without *FR2* shows a greater enhancing effect on crossing-over in the *Gl-Sb* region than the reversed acrocentric with *FR2*, which is clearly what would be expected if exchange frequency were an important factor in interchromosomal effects. However, two facts suggest that these differences are not pertinent to the effect under consideration. First, these tests were all done at different times and no attempt to insure coisogenicity was made, thus rendering small differences between sets of very dubious importance. Secondly, this difference between *RA/O* and *RA/FR2* manifests itself both when the compound carries *In(1)AB* and when it does not. Exchange in reversed acrocentrics carrying *In(1)AB* must, however, be exceedingly low whether or not *FR2* is present[5], making it very unlikely that there is a very big difference in exchange values in the compounds with and without *FR2* in this case.

Finally, it seems not unlikely that reversed acrocentrics without the *AB* inversion and without *FR2* show an overall exchange value not very different from the *In(1)sc*^{8}*/In(1)AB* free-X complex. These two situations, however, show very different interchromosomal effects, again suggesting that exchange in the X *per se* plays but a minor part in influencing crossing-over in the autosomes.

This conclusion supports certain findings of Zimmering[11] who found that autosomal crossing-over was the same in regular and exceptional (*i.e.*, non-disjunctional) progeny of XXY females. Since there is no crossing-over in the X's of exceptional progeny, while there is, of course, in the meiotic cells leading to regular progeny, this again suggests a minor role for exchange in producing interchromosomal effects, although in other inversion combinations, differences between regular and exceptional progeny were found.

Mather's theory on the nature of the interchromosomal effect postulates a defined number of chiasmata per genome[3]. This theory is not substantiated by the results of the present experiment. Were that theory correct, one would expect frequencies of crossing-over in the *Gl-Sb* region to vary inversely with the frequency of exchange in the reversed acrocentric compound. The marked variation in exchange produced by the introduction of a homolog for the reversed acrocentric is not accompanied by the predicted changes in crossing-over in the *Gl-Sb* region.

The complexity of reversed acrocentric behavior and the paucity of information about its precise meiotic mechanics makes it difficult to evaluate other hypotheses about the nature of interchromosomal effects[1, 7], in terms of these results.

It should be pointed out finally that this investigation was designed to test mechanisms which, on the basis of our present knowledge, are considered variables that may influence the interchromosomal effect. Obviously the data do not exclude the possibility that other mechanisms, undefined at present, may be of more fundamental significance in this process.

Summary

Reversed acrocentric compound X-chromosomes show very different distributions of exchange values depending upon whether or not a Y-chromosome is present in the compound-bearing females. It is shown that in spite of this difference in exchange distribution, the interchromosomal effect induced is very nearly the same for the two cases and these are also the same as the interchromosomal effect induced by a comparable free-X inversion combination. These results support the conclusion that the exchange distribution, *per se*, is a minor component of interchromosomal effects on crossing-over.

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THE EFFECT OF ANTIMETABOLITES ON SEX RATIO IN MAMMALS

SIDNEY MITTLER*

ONE-HALF of the young born to mammals are males, and numerous attempts to change this ratio by physical or chemical methods have been unsuccessful. The sex ratio is the result of males producing an equal number of male-determining sperm cells containing Y chromosomes and female-determining sperm cells containing X chromosomes. Since the eggs of mammals contain only X chromosomes, sex is determined by the type of sperm cell that fertilizes the egg. An XX zygote results in a female and an XY a male. Equal production of the two types of sperm cells and equal chances of fertilization by one or the other result in the 1:1 ratio. If the male could be induced to produce more Y sperm cells by either reducing the number of X sperm cells or decreasing their vigor, the chance of producing more males than females would be increased.

Relatively few spermatogonia produce millions of spermatozoa. There is a large demand for nucleic acids in this process, because the nucleus is a very large portion of the sperm and nucleic acids are concentrated in the chromosomes. Inhibition of nucleic acid synthesis by antimetabolites is a logical point of attack, and antimetabolites have been used in this function to repress the growth of cancer cells.

An attempt was made to utilize antimetabolites to upset the normal 1:1 production of X and Y sperms. In this study it was assumed that Y chromosomes contain less nucleic acids than X chromosomes. Therefore, a nucleic acid antimetabolite might favor the production of more Y sperms since fewer or less vigorous X sperms would be produced, resulting in a larger percentage of male offspring. Successful inhibition of X sperm production would not only be the first step by man in controlling the sex of his offspring, but would be an efficient aid in animal husbandry.

Experimental Method

To serve as a control as to sex ratio, male, albino, Swiss mice were each mated to four females for two weeks to obtain offspring not influenced by any drug. Then each male was treated with antimetabolite by intraperitoneal injections five days a week for one month. This length of treatment was necessary so that the sperm cells would be affected in the very early stages of spermatogenesis. Then each male was again mated for three successive periods of 14 days to three different groups of four females. The antimetabolite treatment was continued during these mating periods.

The normal sex ratio of mammalian offspring[5] is one male to one female. A deviation from this ratio with a chi-square of 3.84 is accepted by most biologists as significant. The following equations were used to determine whether the sex ratio was distorted by the drugs:

$$m = \frac{X_1 + X_2}{2}$$

where

m is the expected number of either sex if the ratio is 1:1

X_1 is the number of males

X_2 is the number of females

$$\chi^2 = \frac{(X_1 - m)^2}{m} + \frac{(X_2 - m)^2}{m}$$

The antimetabolites were administered

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at two different levels somewhat below the maximum tolerated dose to determine their influence on the sex ratio. The antimetabolites used were: Amethopterin, 6-purinethiol, 8-azaguanine, N-methylformamide, and Actidione.

Results

The results with all the antimetabolites and the sodium chloride control are listed in Table I.

Amethopterin is a folic acid antagonist which is used to combat leukemia. The maximum tolerated dose for an 11-day treatment of an albino mouse is 0.5 mg/kg per day. One group of male mice was treated with 0.25 mg/kg per day and another group with 0.375 mg/kg per day. Amethopterin had no effect upon the sex ratio.

A triazolopyrimidine analog of guanine, 8-azaguanine acts as an antimetabolite by inhibiting synthesis of the purine needed in the nucleic acid molecule. When this potent antitumor drug was tested for effect upon the sex ratio at 25 mg/kg per day, a chi-square of 4.5, a significant deviation, was obtained

TABLE I. The effect of various antimetabolites on the sex ratio of mice

			Before treatment			During treatment	
Antimetabolite	Dosage, mg/kg/day	Number of males	Females fertilized/total	Sex ratio of litters M/F	Length of treatment, days	Females fertilized/total	Sex ratio of litters M/F
Amethopterin	0.25	6	13/24	39/39	35	16/24	41/43
					45	8/24	18/31
					52	4/24	15/14
							74/88
	0.375	6	17/24	67/51	35	10/12	38/31
					45	6/12	30/28
					52	2/12	4/10
							72/69
8-azaguanine	25	3	6/8	22/23	24[1]	3/8	17/10
					35	2/8	11/4
					48	7/8	31/22
		1	4/4	25/14	21[2]	2/4	7/9
		2	7/8	25/25	35	4/8	16/11
					45	2/8	10/10
					52	4/8	22/18
							114/84
	25[3]	5	18/20	87/78	49	14/20	73/81
					63	11/20	64/76
						14/20	48/58
							185/215
	50	3	5/5	20/21	24[1]	2/4	12/7
					35	3/4	17/8
					48	3/4	[4]
		2	8/8	27/34	35	4/8	12/17
					45	3/8	10/12
					52	5/8	22/20
							73/64

[1] One animal died before being mated for the first time after treatment had started.
[2] Died after being mated for the first time after 21 days of treatment.
[3] Rerun as a check on previous results.
[4] Litters lost before sex determination.
[5] Three animals died before being mated for the first time after treatment had started.
[6] One animal died before being mated for the second time after treatment had started.
[7] One animal was sterile.

(114 male and 84 female offspring). At 50 mg/kg per day there was no significant deviation of the sex ratio, indicating that the previous results were fortuitous. When the experiment with 25 mg/kg per day was repeated, a ratio of 185 males to 215 females was obtained. Therefore, it was concluded that 8-azaguanine has no effect upon the sex ratio of litters fathered by males treated with this drug.

Also interfering with purine synthesis and therefore widely used as an anti-tumor agent is 6-purinethiol hydrate. This drug was too toxic at 25 mg/kg per day, so dosages of 12.5 and 7.25 mg/kg per day were used to test its effect upon the sex ratio. It had no effect.

Although N-methylformamide inhibits Sarcoma 180, its mode of action is unknown. It may act as a metabolic antagonist for single carbon units or through a metabolic by-product produced in the liver. The maximum tolerated dose for a 20-gm. mouse is 175 mg/kg per day for 11 days. Since the male mice were treated five days per week for eight weeks, somewhat lower dosages were used. One group of males was treated with 75 mg/kg per day and another with 125 mg/kg per day; no effect upon the sex ratio was evident.

Actidione, which is 3-[2-(3, 5-dimethyl-2-oxyocyclohexyl)-2-hydroxethyl]-glutarimide, was obtained from the Upjohn Company. It

TABLE I.—Continued

			Before treatment			During treatment	
Antimetabolite	Dosage, mg/kg/day	Number of males	Females fertilized/total	Sex ratio of litters M/F	Length of treatment, days	Females fertilized/total	Sex ratio of litters M/F
6-purinethiol hydrate	7.25	8	26/32	149/118	36	2/4	8/11
					45	7/20	29/27
					60	4/20	16/15
					75	6/20	20/15
							73/68
	12.5	5	19/20	86/94	45[1]	12/16	58/61
					60	13/16	65/80
					75	7/16	48/47
							171/188
N-methylformamide	75	5	18/19	58/57	39	15/20	71/78
					52[6]	11/16	62/59
					66	5/16	18/17
							151/154
	125	5	18/20	70/89	47[1]	9/12	40/41
					60	10/12	54/45
					74	5/8	18/16
		3[7]	4/8	12/17	48	5/8	19/19
					64	4/8	24/22
							155/143
Actidione	7.5	5	18/20	69/85	46[1]	15/16	65/48
					59	15/16	45/51
					74	13/16	30/40
							140/139
	11.2	5	19/20	99/81	43	9/20	47/36
					58	7/20	15/16
					73	9/20	35/43
							97/95
0.9% sodium chloride (control)	0.5 ml/day	5[7]	17/20	71/80	43	13/20	62/56
					57	11/20	47/47
					61	4/20	19/21
			11/16	27/29	46	12/16	64/55
					62	9/16	45/57
			Total	953/935			237/236

is an antibiotic which inhibits Sarcoma 180 in mice at a maximum tolerated dose of 32 mg/kg per day for treatment for seven days. Its mode of action is not clearly understood. The offspring of males treated with 7.5 mg/kg per day and with 11.2 mg/kg per day showed no effect.

Some males were injected with 0.5 ml. per day of 0.9 percent sodium chloride as a control. The 0.5-ml. dose was the maximum amount of fluid injected into any of the mice per day. The repeated injection of this volume of fluid did not affect the sex ratio of the offspring of treated males.

The five anti-tumor drugs used in this study had no effect upon the sex ratio of mice. Although there is probably a difference in the metabolic requirements of a male-producing sperm cell and a female-determining sperm cell, the antimetabolites selected did not favor production or mortality of either one. Further information is required on the basic chemical requirements of synthesis of sperm cells and especially on the different requirements for production of X chromosomes and Y chromosomes.

In this study, it was assumed that Y chromosomes contain less nucleic acids than X chromosomes. This assumption was based on the facts that there are more known genes on X than on Y chromosomes and genes are believed to be larger nucleic acid molecules. Antimetabolites that interfere with nucleic acid metabolism would favor production of Y-chromosome-containing sperm cells. However, since no change was induced in the sex ratio, it can be assumed that equal numbers of X and Y sperm cells were produced.

Twenty years ago, as a result of poorly designed experiments, it was reported that the sex of offspring could be controlled by an acid or alkaline douche of the female before mating. However, Casida and Murphee[1] and McPhee and Eaton[3] demonstrated that neither sodium bicarbonate nor lactic acid has an influence upon which type of sperm cell reaches the egg.

Koltzhoff and Schroder[2] claimed to have used cataphoresis to separate the spermatozoa of rabbits. Females were produced from the sperm collected at the anode. A total of only eight offspring were used to make this report, and the results have not been verified by other research workers.

Another physical method for separation of sperm has been proposed by Newton[4]. It is based on the premise that X and Y chromosomes are different in volume. Thus, the two types of sperm should differ in weight and, according to Newton's calculation, could be separated by the proper centrifugal technique. However, this has never been accomplished.

Summary

An attempt was made to influence the sex ratio of the offspring of mice by treating males with antimetabolites. These anti-tumor drugs were used in an attempt to alter equal production of X- and Y-chromosome-bearing sperm cells. Seventy-four males were injected with the anti-tumor drugs for from 40 to 75 days and mated to 944 females. Amethopterin, 6-purinethiol hydrate, N-methylformamide, Actidione, and 8-azaguanine had no effect upon the sex ratio.

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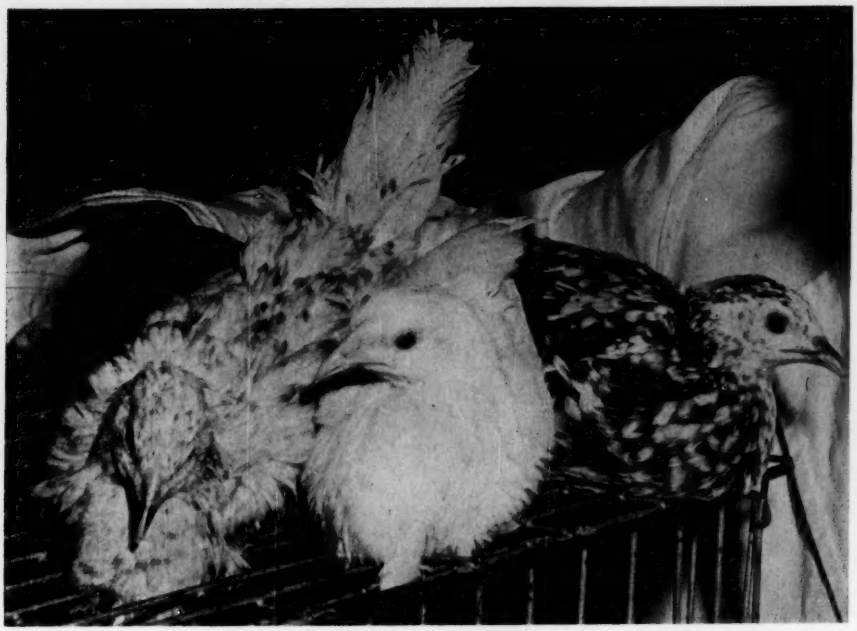
CHICKEN-QUAIL HYBRIDS

F. H. Wilcox and C. Elmer Clark*



CHICKEN-QUAIL HYBRIDS

Figure 13

From left to right, hybrids from Flightless, White Leghorn, and Dark Cornish males at three to four and one-half months of age.

THIS experiment was an attempt to cross the Japanese quail (*Coturnix coturnix japonica*) with the chicken. It appeared to be an interesting cross to make, since these two species belong to the same subfamily (Phasianinae)[6] but to different genera. Hybrids have been hatched from crosses of the chicken to the turkey[5] and the guinea fowl[2], which are members of different families but the same superfamily. Previously, Mitsumoto and Nishida[4] crossed the Japanese quail with the chicken and obtained 5.4 percent fertility but zero hatchability when the chicken male was used, and zero fertility when the quail male was used.

Materials and Methods

Pooled chicken semen was collected, and 0.03 ml. inseminated weekly into each of 20 to 25 quail hens. For insemination the semen was drawn into the end of small glass tubing which was connected with a syringe; the semen was forced out with air in the syringe. Quail hens were separated from quail males at seven weeks of age. They were not in-

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seminated with chicken semen until no fertile eggs had been laid over a period of one week. It was not possible to obtain semen from the quail male, and therefore the cross was made in only one direction. Chicken semen was obtained from three breeds: White Leghorn, a Flightless strain (mostly New Hampshire with dominant white from Leghorn) and year-old and two-year-old Dark Cornish (selected for parthenogenic development). Semen was inseminated immediately after collection, except that Cornish semen was transferred from the Agricultural Research Center at Beltsville, Maryland, which took 10 minutes.

Each week eggs were put into an incubator at 99.75° F., and candled 10 days later; those with living embryos were kept in the incubator 11 days more. All eggs, except those which hatched, were broken out and observed macroscopically for embryonic development. Progeny were fed a turkey starter ration with 250,000 I.U. Vitamin A and 0.6 gm. oleandomycin per 25 pounds.

Results

The results of the inseminations are shown in Table I. Fertility was low, although higher than that previously reported[4]. Ten eggs hatched: two from White Leghorn, five from Dark Cornish, and three from Flightless males. Although hatchability of all eggs was numerically lower for the White Leghorn cross than for the other two breeds, the difference was not statistically significant (chi-square). All chicks which hatched did so without assistance and on the nineteenth day of incubation, with the exception of one which hatched on the eighteenth day and one on the twentieth day. Two of the chicks held their heads to one side, and one (from a White Leghorn male) had crooked toes on one foot; there were no other apparent abnormalities. The down color of the Leghorn and Flightless hybrids was yellow throughout; that of the Cornish hybrids was a brown pattern.

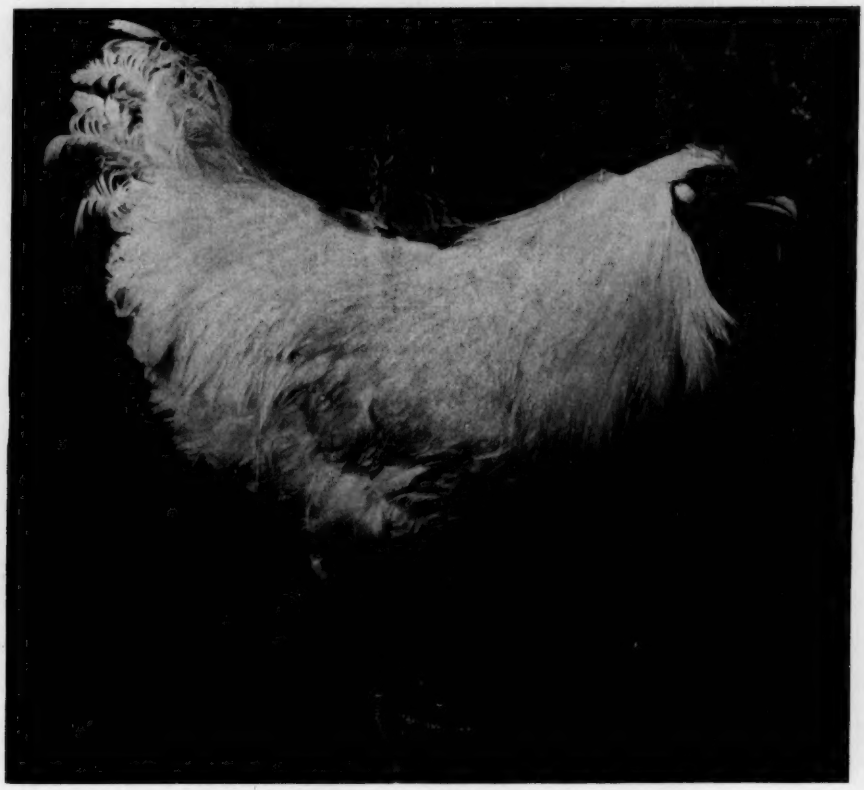
Six of the 10 hybrids died within four days after hatching. The other four lived from four to 11 months of age. One of these was sired by White Leghorn, one by Cornish, and two by Flightless males. Three of them are shown in Figure 13. Plumage was completely white in the Leghorn hybrid, and was white with brown flecks in the two Flightless hybrids; these three were quite wild. The Cornish hybrid had dark brown feathers with some white feathers around the neck region, and was tamer than the other three. The comb was completely absent in all four hybrids, which therefore resembled the quail parents in this respect. Sex was positively identified in only two—from Cornish and Flightless males; both were males.

Weights of the crosses and average weights of the parent stocks are given in Table II. The weight of the male rather than the female is given for the quail, so that all comparisons would be on the same sex; quail hens weighed an average of 22 gm. more than the males. It is obvious that the body weight of the cross is much closer to that of the quail than that of the chicken, even when the data are converted to logarithms. It is note-worthy that there was a great difference in body weight of the two species crossed. This difference is further illustrated in Figure 14.

The first hybrid to hatch was from the White Leghorn male, and suffered

TABLE I. Fertility and hatchability of eggs from quail hens inseminated with chicken semen

Date	Breed of chicken	No. of males	No. eggs	Percent (of all eggs) Dead 1-10 days	Dead 11-20 days	Hatched
Dec.-Jan.	White Leghorn	18	580	5.5	1.2	0.2
Jan.-Feb.	Dark Cornish	11	445	3.4	1.3	0.7
Feb.-Mar.	Flightless	24	513	8.2	1.4	0.6
Mar.-May	White Leghorn	15	471	7.4	0.6	0.2
May	Dark Cornish	10	273	4.8	2.9	0.7
	All breeds	—	2282	6.0	1.4	0.4



STRANGE PARTNERS

Figure 14

Adult quail hen atop an adult Flightless cock.

from pasty vent and slow-growing and ragged feathers. Vitamin A and oleandomycin were added to the feed, and defecation and feather growth became normal. Therefore, these two ingredients were added to the ration for all the other hybrids.

Attempts to collect semen from the hybrids were without success. Daily injections of 0.1 to 0.5 ml. pregnant mare serum (Cutter Labs. ; 50 units/cc.) into the Leghorn hybrid made possible the eversion of the cloaca and production of slight amounts of clear fluid.

TABLE II. Body weight of progeny from quail-chicken cross and of parent stock

		Quail-chicken cross		
		Maximum body wt.		
Parent	Adult body wt. ♂ (gm.)	gm.	Age taken (mo.)	Age at death (mo.)
White Leghorn	2400	335	5	11
Dark Cornish	3600	384	5	7
Flightless	3700	1. 350	3	4
		2. 371	4	9
Quail	102			

Discussion

The low hatchability of eggs from the chicken-quail cross suggests that the two parent species are no more closely related than is the chicken to the guinea fowl or turkey. A similar suggestion was advanced by Mainardi[3] on the basis of his immunological studies and other evidence cited by him.

The most remarkable feature of the present study was the apparent good health and absence of morphological defects in most of the hybrids which hatched. All were highly active, except the two with heads turned to the side. The early death of six of the 10 may possibly have been due to failure to learn to eat or drink, or to rearing in isolation, since in most cases only one hybrid was hatched at a time; but experimental evidence on this is lacking. The average hatching time of 19 days was quite uniform, and is intermediate to that of the quail and chicken, which hatch on the seventeenth[1] and twenty-first day of incubation, respectively. Plumage color was as expected on the basis of genetics of the chicken. Both the White Leghorn and Flightless parents carry dominant white. The presence of many white feathers in the Cornish hybrid cannot be easily explained, since white feathers are absent in both parents.

Summary

Chicken semen of three breeds (White Leghorn, Flightless, Dark Cornish) was inseminated into Japanese quail hens. Ten hybrids (0.4 percent) hatched from 2,282 eggs set, usually on the nineteenth day of incubation. Down color of the Leghorn and Flightless hybrids was yellow and that of the Cornish hybrids was brown. Four of the hybrids lived from four to 11 months. All lacked a comb and were closer to the quail in weight than to the chicken. Plumage was white in the Leghorn hybrid, white with brown flecks in the two Flightless hybrids, and brown with some white in the Cornish hybrid.

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INHERITANCE OF MORPHOLOGICAL ABNORMALITIES IN SEEDLINGS OF TWO SPECIES OF PHASEOLUS

W. P. Bemis and N. Kedar*

CROSSES between species of *Phaseolus* have been made with varying success, often depending upon the varietal lines used as parents. The failures in making successful interspecific crosses can be generally grouped into four categories: (1) failure to obtain fertilization, (2) failure to obtain seed development, (3) failure to obtain normal growth patterns and (4) failure to recover fertility. This paper will concern only the failure to obtain normal growth patterns specifically in the seedling stage of development.

The runner bean, *Phaseolus coccineus*, is used in breeding programs with *Phaseolus vulgaris* as a source of disease resistance and hypogeal germination[2,9]. This interspecific cross is not difficult to make, but may result in the occurrence of seedling abnormalities. The inheritance of these seedling abnormalities was studied and a hypothesis is proposed explaining the genetic action involved.

Hollingshead[6] hypothesized the action of a simple Mendelian factor to be effective in causing a seedling lethal in the interspecific cross *Crepis capillaris* (L.) Wallr. × *C. tectorum* L., which resulted in all seedling lethals, with the exception of those from one *C. tectorum* plant. This particular plant, when crossed with *C. capillaris*, produced progeny that were one-half normal and one-half "dying in the cotyledon stage." The progeny from this plant when crossed to *C. capillaris* segregated into a 1:2:1 ratio, 1 all normal: 2 half normal and half lethal: 1 all lethal. Hollingshead's hypothesis was that the *C. tectorum* plant was genotypically *Ll* and that the *l* allele was lethal in the interspecific cross. No explanation was given for the ineffectiveness of the *l* allele in the parental species.

Lamprecht[7] investigated the genetic barrier between *P. vulgaris* and *P. multiflorus* (*P. coccineus*). He mentions several abnormal interspecific hybrids which appear similar to types discussed in this paper. The interspecific hybrids described by Lamprecht were not uniform, and he suggested that heterogeneity of the F_1 hybrids may be related to the heterozygosity of *P. coccineus* for the self-incompatibility alleles S_1, S_2, S_3, etc.

Morphologically Abnormal Interspecific Seedlings

A line of the variety Scarlet Runner (S.R.) (*Phaseolus coccineus*) maintained at the University of Illinois was used as the male parent in crosses with five varieties of *P. vulgaris*. All of the crosses were made following hand pollination without emasculation techniques described by Buishand[4]. Genetic markers were used to distinguish the crosses from the selfs. The frequency of accidental selfs in the hand-pollinated populations was very low, comprising less than one percent of the total crosses made. The hand-pollinated seeds were planted in a sand bench in the greenhouse for observation of seedling development. Selfed seeds of the parental lines were planted as checks for added precaution in identifying accidental selfs in the hand-pollinated populations. Three different types of seedlings were noted in the F_1 interspecific hybrids (Table I, Figure 15). Each of the five F_1 seedling populations was uniform.

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ABNORMAL INTERSPECIFIC SEEDLINGS
Figure 15

A—Seedlings of *P. vulgaris* variety Blue Lake on the left, *P. coccineus* variety Scarlet Runner on the right, and the F_1 interspecific B-dwarf hybrids in the center. *B*—Seedlings of *P. vulgaris* variety Tendergreen on the left, *P. coccineus* variety Scarlet Runner on the right. and the F_1 interspecific T-dwarf hybrids in the center.

When the variety Canocel (Can.) was used as the *vulgaris* parent, the seedlings were all normal in growth. The *vulgaris* variety Blue Lake (B.L.), when used as a parent, produced an F_1 population that was uniform for a B-type morphological dwarf. The percent of germination for populations of the B dwarf was 62.2 percent for a total of 74 seeds planted. This type of abnormal seedling was essentially lethal in that it rarely survived through the development of the first trifoliate leaf, and never past the development of the third trifoliate leaf. The leaves were smooth but restricted in size, and even though the seedling remained alive for several weeks, the cotyledons remained unabsorbed.

The *vulgaris* varieties Tendergreen (Tend.), Light Red Kidney (L.R.K.) and White Marrow (W.M.) when crossed with Scarlet Runner produced similar F_1 populations. These populations were uniform for a T-type morphological dwarf. The leaves of these hybrids were restricted in size, rugose, and stippled with brown spots. The stems were also stippled. The cotyledons were absorbed and growth continued in a restricted manner. This type of morphologically abnormal plant produced flowers that were normal in size containing viable pollen. Growth of the plants of the T-type dwarf has been maintained for over a year. They continue to flower, but are incapable of producing seed. The percent of germination for populations of the T dwarf was 94.7 percent for a total of 133 seeds planted.

Three-Allele, Two-Loci Hypothesis

Since the apparently uniform *coccineus* parent was common to all of the crosses, it appeared that the genetic differences resulting in the three types of F_1 seedlings must come from the *vulgaris* varieties. Crosses between *vulgaris* varieties, however, were all normal in seedling development in the F_1 and F_2 generations. This indicated that the *vulgaris* varieties were compatible, and only when crossed with *coccineus* would incompatibility have occurred, resulting in the abnormal seedling development. The occurrence of two types of abnormal seedlings designated T dwarf and B dwarf raised the question of whether the manifestation of the dwarfs in the F_1 interspecific populations was the result of an allelic series or of two independent loci. To test these hypotheses the F_1 plants of the *vulgaris* varietal crosses were crossed with a common *coccineus* parent (Table II).

It is apparent from the data in Table II that the T dwarf and B dwarf are the result of the action of two independent loci. In addition to T dwarfs and B dwarfs, a type of abnormal seedling designated TB dwarf was observed. TB dwarfs were the size of B dwarfs, or smaller. Their cotyledons remained unabsorbed as in B dwarfs, and they manifested the rugose leaves and brown stippling of T dwarfs. The four types of seedling development, including the occurrence of normal seedlings in crosses 6, 7, and 9 (Table II), indicate the genetic action of two loci. The 1:1:1:1 ratio (Table III) substantiates the hypothesis.

The genetic action resulting in the T dwarfs (Table I) for the *vulgaris* varieties Tendergreen, Light Red Kidney and 7-16 White Marrow was assumed to be the result of a common allele, as indicated in the data for crosses 10, 11, and 12 (Table II). There was no evidence of segregation for types of seedling development other than the T dwarf, indicating the *vulgaris* parent (F_1 varietal cross) was homozygous for the allele producing the T dwarf. The action of three alleles at two independent loci was hypothesized to explain the data in Tables II and III. The two loci are designated *T* and *B* after the type of abnormal dwarfs produced. At each locus there are three possible alleles. These are designated by the superscripts *v*, *vc*, and *c* (*v* = *vulgaris*, *c* = *coccineus*). The allele *v* is compatible with *v* or *vc*, but is incompatible with *c*, producing the abnormal seedling effect of

TABLE I. The occurrence of three types of seedling development in F_1 populations of different varieties of *P. vulgaris* × a common variety of *P. coccineus*

Cross no.	Parental varieties *P. vulgaris* × *P. coccineus*	F_1 seedling progenies Normal	T dwarf	B dwarf
1	Canocel × Scarlet Runner	9	—	—
2	Blue Lake × Scarlet Runner	–	—	46
3	Tendergreen × Scarlet Runner	–	88	—
4	Light Red Kidney × Scarlet Runner	–	18	—
5	White Marrow × Scarlet Runner	–	20	—

TABLE II. The occurrence of four types of seedling development in populations between F_1 *P. vulgaris* varietal crosses and a common variety of *P. coccineus*

Cross no.	Parental lines *P. vulgaris* × *P. coccineus*	Seedling progenies Normal	T dwarf	B dwarf	TB dwarf	Unknown*
6	(Tend. × B.L.) F_1 × S.R.	28	26	28	29	4
7	(L.R.K. × B.L.) F_1 × S.R.	10	9	9	4	2
8	(B.L. × W.M.) F_1 × S.R.	8	4	11	4	–
9	(B.L. × Can.) F_1 × S.R.	6	—	3	—	–
10	(Tend. × L.R.K.) F_1 × S.R.	—	6	—	—	–
11	(Tend. × W.M.) F_1 × S.R.	—	9	—	—	–
12	(L.R.K. × W.M.) F_1 × S.R.	—	13	—	—	–

*Abnormal seedlings which could not be classified.

that particular locus. Likewise, the allele *c* is compatible with *c* or *vc* but is incompatible with *v*, and the allele *vc* is compatible with *v*, *c*, or *vc*.

The assigned genotypes for the varieties, parental lines and progenies of the *Phaseolus* species are shown in Table IV. The variety Scarlet Runner (*P. coccineus*) has the genotype $T^cT^cB^cB^c$. The variety Canocel (*P. vulgaris*), which is compatible with Scarlet Runner, has the genotype $T^{vc}T^{vc}B^{vc}B^{vc}$. The variety Blue Lake (*P. vulgaris*), which produces B dwarfs when crossed with Scarlet Runner, is incompatible at the *B* locus and has the genotype $T^{vc}T^{vc}B^vB^v$. The other *P. vulgaris* varieties (Tendergreen, Light Red Kidney, and White Marrow) that produce T dwarfs when crossed with Scarlet Runner are incompatible at the *T* locus and have the genotype $T^vT^vB^{vc}B^{vc}$.

In order to test the proposed hypothesis, a series of crosses was made between *P. vulgaris* lines and T dwarfs, and between *P. vulgaris* lines and normal segregates from cross 6, (Tendergreen × Blue Lake) F_1 × Scarlet Runner. The observed and expected results with chi-square and probability values are shown in Table V.

The data fit the proposed hypothesis except for cross 15, Blue Lake × T dwarf (Tend. × S.R.) F_1. This cross is the same as cross 14 but was made at a later date. The seed from cross 14 was planted on April 9 and the seedlings were classified during the period from April 28 through May 28. The seed from cross 15 was planted on July 1 and the seedlings were classified during the period from July 9 through July 22. Possible explanations for the occurrence of T- and TB-type dwarfs in cross 15 are that different parent plants were used and the *vulgaris* parent Blue Lake was incompatible at both the *T* and the *B* locus. It is also possible that there are present additional alleles at other loci which produce a seedling abnormality similar to the T dwarf. Another possibility might be insect contamination

TABLE III. Goodness of fit to a 1:1:1:1 ratio of four types of seedling development in populations between F_1 *P. vulgaris* varietal crosses and a common variety of *P. coccineus*. Cross numbers refer to Table II

Cross no.		Seedling progenies: Normal	T dwarf	B dwarf	TB dwarf	χ^2	P
6	Obs.	28	26	28	29	.171	.98–.99
	Exp.	27.75	27.75	27.75	27.75		
7	Obs.	10	9	9	4	2.750	.30–.50
	Exp.	8	8	8	8		
8	Obs.	8	4	11	4	5.148	.10–.20
	Exp.	6.75	6.75	6.75	6.75		
Pooled	Obs.	46	39	48	37	2.000	.50–.70
	Exp.	42.5	42.5	42.5	42.5		

TABLE IV. Genotypes for the varieties, varietal lines and progenies based on a two-loci, three-allele system. Cross numbers refer to Tables I and II

Cross no.	Parental lines: *P. vulgaris* × *P. coccineus*		Seedling progenies: Normal	T dwarf	B dwarf	TB dwarf
1	$T^{vc}T^{vc}B^{vc}B^{vc}$ × $T^cT^cB^cB^c$		$T^{vc}T^cB^{vc}B^c$	—	—	—
2	$T^{vc}T^{vc}B^vB^v$ ×	"	—	—	$T^{vc}T^cB^vB^c$	—
3	$T^vT^vB^{vc}B^{vc}$ ×	"	—	$T^vT^cB^{vc}B^c$	—	—
4	$T^vT^vB^{vc}B^{vc}$ ×	"	—	$T^vT^cB^{vc}B^c$	—	—
5	$T^vT^vB^{vc}B^{vc}$ ×	"	—	$T^vT^cB^{vc}B^c$	—	—
6	$T^vT^{vc}B^vB^{vc}$ ×	"	$T^{vc}T^cB^{vc}B^c$	$T^vT^cB^{vc}B^c$	$T^{vc}T^cB^vB^c$	$T^cT^cB^vB^c$
7	$T^vT^{vc}B^vB^{vc}$ ×	"	$T^{vc}T^cB^{vc}B^c$	$T^vT^cB^{vc}B^c$	$T^{vc}T^cB^vB^c$	$T^vT^cB^vB^c$
8	$T^vT^{vc}B^vB^{vc}$ ×	"	$T^{vc}T^cB^{vc}B^c$	$T^vT^cB^{vc}B^c$	$T^{vc}T^cB^vB^c$	$T^vT^cB^vB^c$
9	$T^{vc}T^{vc}B^vB^{vc}$ ×	"	$T^{vc}T^cB^{vc}B^c$	—	$T^{vc}T^cB^vB^c$	—
10	$T^vT^vB^{vc}B^{vc}$ ×	"	—	$T^vT^cB^{vc}B^c$	—	—
11	$T^vT^vB^{vc}B^{vc}$ ×	"	—	$T^vT^cB^{vc}B^c$	—	—
12	$T^vT^vB^{vc}B^{vc}$ ×	"	—	$T^vT^cB^{vc}B^c$	—	—

of the hand-pollinated flowers. However, other hand-pollinated crosses made during the same period did not show any indication of contamination.

The lack of TB seedlings in crosses 22, 23, and 24 could be explained in that the expected numbers were so low that they might be missing due to chance alone, but this type of dwarf is weaker than either the T or B dwarfs and it is quite likely that many seedlings of this type fail to develop in the embryo stage, thus producing aborted seeds. It was also difficult to classify the TB dwarfs as such. In cross 22 there were four abnormal seedlings that remained unclassified. The chi-square value for the pooled data from crosses 22, 23, and 24 is 11.391, which has a p value of $<.01$.

A random sample of 16 F_2 plants from the cross Blue Lake × Tendergreen were crossed with the *P. coccineus* variety Scarlet Runner (Table VI). The 16 F_2 plants were also selfed, and the F_3 population from each F_2 plant was normal for seedling development.

A new source of Scarlet Runner obtained from the Joseph Harris Seed Company was used in addition to the Illinois line, and was found to be heterozygous or heterogeneous at the *T* locus. The Harris Scarlet Runner, when crossed with Blue Lake, produced all B-type dwarfs. However, when crossed with Tendergreen, Light Red Kidney or White Marrow, normal plants as well as T dwarfs were produced (6 normal : 20 T dwarf). Which plant or plants of the Harris Scarlet Runner was responsible for the normal segregates in these populations is unknown. Several plants of the new source were used as male parents, and no record was kept of which carried the T^{te} allele instead of the T^{e} allele. Since Scarlet Runner is cross-pollinated, it is quite likely that heterozygous plants

TABLE V. Progenies of *P. vulgaris* lines × T dwarfs and *P. vulgaris* lines × normal segregates from the cross (Tendergreen × Blue Lake)F_1 × Scarlet Runner. The chi-square and probability values for the goodness of fit to the expected ratios are shown

Cross no.	Parental lines				Normal	T dwarf	B dwarf	TB dwarf	χ^2	P
13	Tend.	×	(Tend. × S.R.) F_1 T dwarf	Obs.	34	31			.138	.70–.80
				Exp.	32.5	32.5				
14	B.L.	×	"	Obs.	10		8		.222	.50–.70
				Exp.	9		9			
15	B.L.	×	"	Obs.	15	6	6	1	—	—
				Exp.	14	–	14	–		
16	(Tend. × B.L.) F_1	×	"	Obs.	20	7	1	1	5.268	.10–.20
				Exp.	16.3	5.4	5.4	1.8		
17	(Tend. × L.R.K.) F_1	×	"	Obs.	2	5			1.285	.20–.20
				Exp.	3.5	3.5				
18	(Tend. × W.M.) F_1	×	"	Obs.	6	2			2.000	.10–.20
				Exp.	4	4				
19	(L.R.K. × W.M.) F_1	×	"	Obs.	3	1			1.000	.30–.50
				Exp.	2	2				
20	Tend.	×	[(Tend. × B.L.) F_1 S.R.] normal segregates	Obs.	3	9			3.000	.05–.10
				Exp.	6	6				
21	B.L.	×	"	Obs.	6		7		.077	.70–.80
				Exp.	6.5		6.5			
22	(Tend. × B.L.) F_1	×	"	Obs.	21	11	18	0	10.942*	.02–.01
				Exp.	28.1	9.4	9.4	3.1		
23	(L.R.K. × B.L.) F_1	×	"	Obs.	3	2	3	0	2.667	.30–.50
				Exp.	4.5	1.5	1.5	.5		
24	(B.L. × W.M.) F_1	×	"	Obs.	11	4	3	0	1.359	.70–.80
				Exp.	10.1	3.4	3.4	1.1		
25	(B.L. × Can.) F_1	×	"	Obs.	1		1		.667	.30–.50
				Exp.	1.5		.5			

*Yates correction.

are common. However, the crosses in Table VI were made before it was known that the plants were not homozygous or homogeneous for the T^vT^v genotype.

Since the Harris Scarlet Runner was not homozygous or homogeneous for the *T* locus and the individual plants used as parents in the crosses were not recorded, it is not possible to determine the expected genotypes of the F_2 plants (Blue Lake × Tendergreen) in Table VI. However, the data in Table VI are given as they support the proposed two-loci, three-allele hypothesis. The 11 (Tend. × B.L.) F_2 plants that were crossed with Illinois Scarlet Runner are a random sample, and the pooled data should fit a 1:1:1:1 segregation for the four types of seedling development. The observed numbers for each phenotype are 14:12:10:8, which when tested for goodness of fit have a chi-square value of 1.818 and a probability value of .50-.70.

There are 15 possible combinations of phenotypes when four independent phenotypic classes make up the segregating populations. However, only nine combinations are expected based on the two-loci, three-allele hypothesis (Table VII). The data in Table VI show that five combinations of phenotypes were observed, and all of these were combinations that were expected. For example, normal segregates should occur in four possible combinations—all normal, normal : T dwarf, normal : B dwarf, and normal: T dwarf: B dwarf: TB dwarf. The 12 populations in which normals appeared (Table VI) were in expected combinations.

Evidence of Linkage Between the *D* and *T* Loci

Scarlet Runner is an indeterminant type plant, and this character is controlled by a monogenic dominant *D/d* factor in *P. lunatus*[1] and *P. vulgaris*[5]. The *P. vulgaris* varieties Tender-

TABLE VII. Frequency of genotypes of the F_2 population from the cross Blue Lake × Tendergreen and the expected phenotypic seedling segregation when crossed with Illinois Scarlet Runner

Ratio	Genotype	Seedling phenotype
1	$T^vT^vB^vB^v$	TB
2	$T^vT^vB^vB^{vc}$	1 TB : 1 T
1	$T^vT^vB^{vc}B^{vc}$	T
2	$T^vT^{vc}B^vB^v$	1 TB : 1 B
4	$T^vT^{vc}B^vB^{vc}$	1 TB : 1 T : 1 B : 1 normal
2	$T^vT^{vc}B^{vc}B^{vc}$	1 T : 1 normal
1	$T^{vc}T^{vc}B^vB^v$	B
2	$T^{vc}T^{vc}B^vB^{vc}$	1 B : 1 normal
1	$T^{vc}T^{vc}B^{vc}B^{vc}$	normal

TABLE VI. Segregation for seedling development in the progenies of (Blue Lake × Tendergreen) F_2 × either Illinois Scarlet Runner or Harris Scarlet Runner

(B.L. × Tend.)F_2 Plant no.	Normal		T dwarf		B dwarf		TB dwarf			Types
	I.S.R.*	H.S.R.†	I.S.R.	H.S.R.	I.S.R.	H.S.R.	I.S.R.	H.S.R.	Total	segregating
1	1	8	3						12	N,T
2	1	5	1	7					14	N,T
3						9	1		10	B,TB
4					1	6			7	B
5	2	12	2	1					17	N,T
6		19		1					20	N,T
7		15							15	N
8		12	2						14	N,T
9	5	11	2						18	N,T
10	1	6	1	4	2	1	3	2	20	N,T,B,TB
11						8			8	B
12		5							5	N
13		3		1		1	1		6	N,T,B,TB
14		19							19	N
15	4	10	1		1	5	3		24	N,T,B,TB
16					6	11			17	B
Pooled	14	125	12	14	10	41	8	2	226	

*Illinois Scarlet Runner
†Harris Scarlet Runner

green, Light Red Kidney, and White Marrow are all determinant types. The interspecific hybrids between these three varieties and Illinois Scarlet Runner were all T dwarfs and were all indeterminant or not classified as to plant type due to abnormal seedling growth. There were no determinant F_1 T-dwarf hybrids classified in any of the populations. The F_1 T-dwarf hybrids were back-crossed to the *P. vulgaris* varieties or varietal crosses and segregated normal and T-dwarf seedlings in a 1:1 ratio (Table V, crosses 12, 17, 18, 19). Thirty-five of the normal segregates were classified according to plant type and the data are shown in Table VIII. The T-dwarf segregates were not maintained long enough to be classified as to determinant or indeterminant plant type. The expected ratio of determinant to indeterminant plants in the normal segregates is 1:1 if the *D* locus and *T* locus are independent. However, the data in Table VIII do not fit this expected ratio, having more determinant plants than expected. An aberrant ratio of this type is indicative of linkage between the *D* and *T* loci. Following the "Method of Maximum Likelihood"[8] the calculated linkage value for the *D* and *T* loci is 14.28 ± 5.91 percent recombination (Table VIII).

Linkage between the *D* locus and a gametophytic locus (*Ga*) which causes selective fertilization and results in aberrant ratios of determinant and indeterminant plant types has been reported in lima beans[3]. A linkage of this nature, if occurring in the crosses shown in Table VIII, would cause a similar aberrant ratio of determinant and indeterminant plant types in the T-dwarf segregates. Linkage between the *D* and *T* loci would cause an aberrant ratio of determinant and indeterminant plant types in the T-dwarf segregates that would have an excess of indeterminant plant types. Since the T-dwarf segregates were not classified, the possibility of a *D/GA* linkage occurring in the crosses shown in Table VIII cannot be resolved. The linkage calculations whether for $d/T^v\ D/T^e$ or for $d/Ga\ D/ga$ would be the same based on the data in Table VIII.

Discussion

A high degree of relationship exists between the species *P. vulgaris* and *P. coccineus*, as indicated not only by the similarity of plant, flower, and fruit character, but especially by the relative ease with which interspecific crosses can be made. However, as the species remain isolated, genetic differences arise that tend to block further crossing between them. Plant breeders are concerned with bridging these genetic blocks to create cross-compatible lines so that the desirable traits of various species can be incorporated into a single line.

Five varieties of *P. vulgaris* were crossed with a common *P. coccineus* parent. One *P. vulgaris* variety (Canocel) was found to be compatible for normal seedling development. Two types of abnormal seedlings designated T and B dwarfs were produced by the other four *vulgaris* varieties. One variety (Blue Lake) produced B dwarfs, and the other three (Tendergreen, Light Red Kidney, and White Marrow) produced T dwarfs. All five of the *P. vulgaris* varieties are compatible when crossed with each other. Therefore, Canocel is a naturally occurring bridge between *P. coccineus* and the *P. vulgaris* varieties which are incompatible with *P. coccineus*. If a variety such as Canocel was not available, then by understanding the inheritance of the morphological seedling blocks, a *P. vulgaris* line could be synthesized that would act as a cross-compatible bridge between the two

TABLE VIII. Deviation from the expected 1:1 ratio for determinant:indeterminant plant type in the normal seedling segregates from the indicated crosses. The calculated linkage between the *D* and *T* loci is shown

Cross no.	Parental lines		Normal segregates Determinant	Indeterminant	χ^2	P
13	Tend. × (Tend. × S.R.)F_1	Obs.	22	3	12.960*	>.01
		Exp.	12.5	12.5		
17-18-19	(*vul.* × *vul.*) ×	Obs.	8	2	2.500*	.10–.20
	(Tend. × S.R.)F_1	Exp.	5	5		
	Pooled	Obs.	30	5	16.456*	>.01
		Exp.	17.5	17.5		

*Yates correction. Linkage calculated for $d/T^v\ D/T^e$ from the pooled data = 14.28 ± 5.91 percent recombination.

species. The *P. vulgaris* varieties Blue Lake and Tendergreen, when crossed with Illinois Scarlet Runner (*P. coccineus*), produced progeny that were entirely abnormal, B and T dwarfs, respectively. When the F_1 of the *P. vulgaris* varieties was crossed with *P. coccineus*, one quarter of the progeny was normal. The remainder of the progeny consisted of T, B, and TB dwarfs. This led to the two-loci, three-allele hypothesis. Based on this hypothesis, the proposed genotype for *P. coccineus* (Scarlet Runner) was $T^cT^cB^cB^c$. The genotypes for Blue Lake and Tendergreen were $T^{vc}T^{vc}B^vB^v$ and $T^vT^vB^{vc}B^{vc}$, respectively. Therefore, a cross-compatible line would have the genotype $T^{vc}T^{vc}B^{vc}B^{vc}$ as was the case with the variety Canocel. The Blue Lake × Tendergreen F_1, having the genotype $T^vT^{vc}B^vB^{vc}$, will segregate F_2 plants that will in turn produce nine different progeny segregations when crossed with the Illinois line of Scarlet Runner (Table VII). One-sixteenth of the F_2 population will be cross-compatible, having the genotype $T^{vc}T^{vc}B^{vc}B^{vc}$.

A system of three alleles as hypothesized here would explain the results of Hollingshead[6] and account for the ineffectiveness of the *l* allele in the parental species. The particular *C. tectorum* plant in Hollingshead's investigation would have one allele that was cross-compatible with *C. capillaris* and one allele compatible only with *C. tectorum* ($L^{cl}L^l$). *C. capillaris* would have the genotype L^cL^c, and with the exception of the particular plant mentioned, *C. tectorum* would have the genotype L^lL^l. The alleles L^c and L^l would be incompatible and result in the seedling lethal. The allele L^{cl} would be compatible with the alleles L^c, L^l, and L^{cl}.

The nature of the genetic incompatibility between certain varieties of *P. vulgaris* and *P. coccineus* presented in this paper is one of abnormal seedling development. There is no *a priori* reason that similar genetic incompatibility between these species should be restricted to seedling development and not occur as embryo lethals, or fertility barriers. The nature of the inheritance of the seedling incompatibility presented here suggests that more success in obtaining viable interspecific crosses would be expected when intraspecific varietal crosses rather than straight varietal parents are used.

Summary

Five varieties of *P. vulgaris* were crossed with a common variety of *P. coccineus* to study the inheritance of abnormal seedling development. The *P. vulgaris* varieties were Blue Lake, Canocel, Light Red Kidney, Tendergreen, and White Marrow. The *P. coccineus* variety was Scarlet Runner. Canocel was compatible with Scarlet Runner. Blue Lake was incompatible with Scarlet Runner, producing B dwarfs which were seedling lethals. Light Red Kidney, Tendergreen, and White Marrow were incompatible with Scarlet Runner, producing T dwarfs which developed to the extent that flowers and viable pollen were produced. The five *P. vulgaris* varieties were compatible with each other. A series of three alleles at two independent loci was proposed to explain the inheritance of the dwarf seedlings. The alleles were designated by the superscripts *v*, *vc*, and *c* for each loci. At the *B* locus B^v is incompatible with B^c, producing B dwarfs. B^{vc} is compatible with B^v or B^c. The same hypothesis is proposed for the *T* locus. The cross-compatible genotype between *P. vulgaris* and *P. coccineus* would be $T^{vc}T^{vc}B^{vc}B^{vc}$, as was the case with the variety Canocel. Evidence of linkage between the *D* locus and the *T* locus with 14.28 ± 5.91 percent recombination is presented.

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INHERITANCE OF DIELDRIN RESISTANCE IN THE HOUSEFLY

MOHAMMAD ABDULLAH*

IT HAS been stated that resistance to BHC and cyclodiene insecticides is genetically of different origin from DDT resistance[1]. Transmission of resistance to chlordane in the housefly has been found to be polygenic in nature[4,5]. A multifactorial origin is reported for the BHC resistance of the housefly by Busvine and Khan[2]. The object of this paper is to report on the mode of inheritance of resistance to Dieldrin.

Materials and Methods

The resistant strain (R) of flies employed in this investigation has been reared in Dr. Kearns' laboratory for many years and originally came from the Public Health Service laboratory in Savannah, Georgia. The susceptible strain (S) of flies used is the Staufer strain, obtained from Staufer Chemical Company, Richmond, Virginia.

By single-pair, reciprocal mating of male and female parents (P) of susceptible and resistant origin, an F_1 generation was obtained, a portion of which was back-crossed with the susceptible parent strain to produce an F_2 generation and the rest used in dosage-mortality tests. All the individuals of the F_2 generation were tested for their level of resistance to Dieldrin.

The flies were individually treated by topical application with a microsyringe of different concentrations of Dieldrin in acetone solution. Mortality was determined after 24 hours.

Results

The percent of mortality of resistant and susceptible strains of flies is plotted in Table I and illustrated in Figure 16. Detailed results of tests on groups of female and male flies in the F_1 and F_2 generations are given in Tables II and III, respectively, and also illustrated in Figure 16. They are summarized in Table I. It should be noted that the

TABLE I. Percentage of mortality of flies with Dieldrin in micrograms per fly

	.005	.01	.02	.06	.1	.5	1	2	5	10	25	50	100	150	200
Susceptible males*	8	14	24	31	40	52	75	100	100	100					
Susceptible females*	2	2	9	21	24	47	64	100	100	100					
Resistant males*									0	0	30	41	100	100	100
Resistant females*									0	0	20	33	70	80	82
F_1 males 1-12					*0*	*7*	*12*	*22*	*36*	*57*	*79*	*98*	*100*		
F_1 females 1-12					0	5	8	19	19	35	53	78	84		
F_2 males 13-22					10	15	21	26	40	59	100	100	100		
F_2 females 13-22					0	0	0	0	10	48	55	90	100		

*One hundred flies were used in each test.

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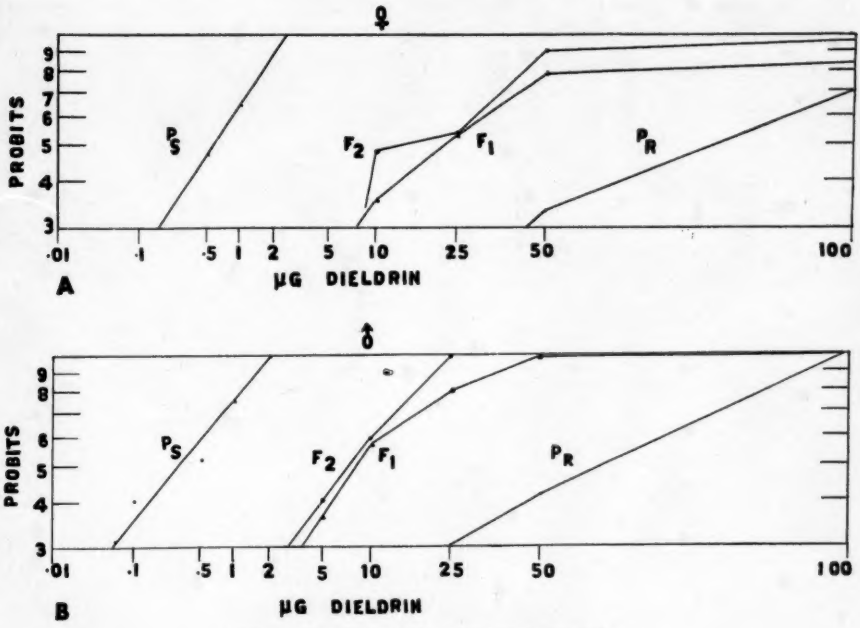
TABLE II. Results of tests on groups of female flies in the progenies of various susceptible × resistant cross-matings

Generation	Original cross Type	Pair no.	Proportions of flies killed by different concentrations of Dieldrin .1μg	.5μg	1μg	2μg	5μg	10μg	25μg	50μg	100μg
F_1	S ♂ × R ♀	1-7	0	4	4	18	20	32	54	74	54
	total		6	84	70	94	110	92	100	96	58
F_1	S ♀ × R ♂	8-12	0	2	5	7	10	9	9	14	9
	total		20	29	40	29	46	26	20	17	10
F_2	S ♂ × F_1 ♀	13-17	0	0	0	0	3	16	12	35	23
	total		15	20	20	20	30	35	20	40	23
F_2	S ♀ × F_1 ♂	18-22	0	0	0	0	3	13	21	37	25
	total		15	20	20	20	30	25	40	40	25
F_1	all data	1-12	0	6	9	25	30	41	63	88	63
			26	113	110	133	156	118	120	113	68
F_2	all data	13-22	0	0	0	0	6	29	33	72	48
			30	40	40	40	60	60	60	80	48

TABLE III. Results of tests on groups of male flies in the progenies of various susceptible × resistant cross-matings

Generation	Original cross Type	Pair no.	Proportions of flies killed by different concentrations of Dieldrin .1μg	.5μg	1μg	2μg	5μg	10μg	25μg	50μg	100μg
F_1	S ♂ × R ♀	1-7	0	8	8	20	32	50	64	80	54
	total		4	90	70	94	110	100	80	82	54
F_1	S ♀ × R ♂	8-12	0	0	4	8	19	11	20	8	15
	total		20	22	30	34	32	24	26	8	15
F_2	S ♂ × F_1 ♀	13-17	3	4	7	9	12	20	25	25	25
	total		30	30	35	35	30	35	25	25	25
F_2	S ♀ × F_1 ♂	18-22	2	5	9	8	12	15	15	18	22
	total		20	30	40	30	30	25	15	18	22
F_1	all data	1-12	0	8	12	28	51	61	84	88	69
			24	112	100	128	142	124	106	90	69
F_2	all data	13-22	5	9	16	17	24	35	40	43	47
			50	60	75	65	60	60	40	43	47



DISTRIBUTION OF SUSCEPTIBILITY

Figure 16

Distribution of susceptibility to Dieldrin in colonies of susceptible (S) and resistant (R) houseflies and in progeny of crosses between them, as shown by log concentration-probit regression lines among females and males, respectively.

LD_{50} value for resistant flies is about 100 times that for the normal strain of flies.

There is no evidence for a sex-linked factor in inheritance either in the F_1 or the F_2 progenies of crosses between male and female parents of susceptible or resistant origin. Therefore, the results of reciprocal crosses between both types of pairs have been added together and plotted for males and females separately. There is no segregation in the F_2 generation, which indicates multifactoriality. The F_1 generation is intermediate in resistance between the parents. Some of the F_1 and F_2 females, however, approach the level of resistance of the resistant strain. The F_2 generation is also intermediate in resistance, but at a slightly lower level than the F_1. This is similar to the findings of Busvine and Khan[2] with BHC.

Discussion

The best criterion of isolation of a major gene is by repeated backcrossing with selection[7]. Crow[8] mentions, "It is customary in quantitative characters to assume that any trait that does not lead to Mendelian segregations is polygenic. I suppose that polygenic means any number of factors larger than can be individually identified. . ." According to Mather[6], an intermediate F_1 generation with narrow variation, followed by wide variation in the F_2, indicates multifactoriality. In view of these comments, the results obtained indicate a polygenic inheritance of resistance to Dieldrin in the housefly.

Summary

A polygenic origin for the Dieldrin resistance of the housefly, *Musca domestica* Linnaeus, is indicated by the results obtained using single pairs and topical application of the in-

secticide. There is no evidence for a sex-linked factor.

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INSTITUTE FOR SCIENTIFIC INFORMATION

RESEARCH scientists will soon be consulting a more precise and specific literature index that links together subject material that would never be collated by usual indexing systems. Concerned with new starting points for scientific literature searches, the unique concept uncovers sometime-buried associations, relating important works and authors, yet keeps the researcher abreast of the masses of current published scientific information. This new approach to information retrieval is called the Citation Index.

A $300,000 grant extending over a three-year period has been awarded to the Institute for Scientific Information, Philadelphia, Pennsylvania, to study the practicability of citation indexes and to test their techniques of preparation. The project, under joint sponsorship of the National Institutes of Health and the National Science Foundation, is aimed at producing a unified citation index for science including the publication of a genetics index.

Dr. Eugene Garfield, director of ISI, explains the simplified citation index this way. If this article you are now reading were processed for citation indexing, it would be called the "referant." All the items in the bibliography would be called "references." A list of these references, each of which is followed by its associated list of referants, becomes the citation index. By using a citation index, the researcher finds out what works have cited a particular reference following its publication.

By focusing on the individual citation rather than specific subjects, the citation index signifies a more sophisticated method of scientific documentation, as well as a growing bibliographical aid. Because the scientific researcher is generally aware of one or more particular papers already published in the area of his specialized interests, he will use the citation index as a starting point, rather than the specific topics found in conventional indexing.

Better scientist-to-scientist communication is expected, for authors can see at a glance what literature has been published since their works, in their own and related fields, that refer back to their own works.

By virtue of its different construction and purpose, the citation index complements indexes like Beilstein, Chemical Abstracts, Biological Abstracts and is not intended as a substitute.

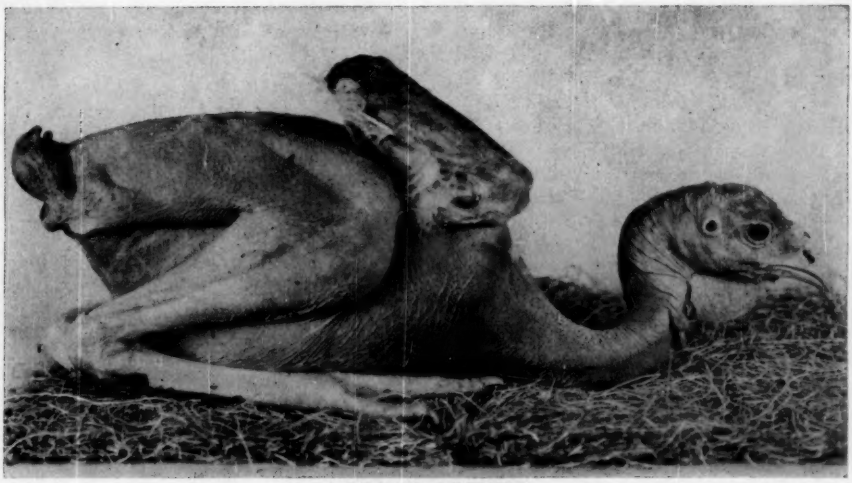
"Experimental studies on systems for extracting and processing citations will soon be completed," Dr. Garfield reports. It is estimated that approximately one million references will be processed during the next six months on a high speed magnetic tape computer. According to Dr. Garfield, "approximately three million citations, from scientific literature published in the five-year period 1959 to 1963, will be processed during the life of the project."

As a guide to the project and aid towards refining concepts of the methodology, an advisory committee of scientists has been established. Members of this board are Dr. Gordon Allen, National Institute of Mental Health; Dr. Joshua Lederberg, Stanford University; Dr. George LeFevre, Harvard University; Dr. Joseph Melnick, Baylor University; Dr. Sol Spiegelman, University of Illinois.

AN AUTOSOMAL NAKED MUTATION AND ASSOCIATED POLYDACTYLISM

in Beltsville Small White Turkeys

H. K. Poole and S. J. Marsden*



A NAKED MUTANT

Figure 17

Homozygous recessive autosomal naked mutant in the Beltsville Small White turkey. This male bird is 12 weeks old. The skin has no feathers or feather follicles except on the caudal margin of each wing and two contour feathers on the right shoulder. Note the complete absence of scales on the shank and foot.

A RECESSIVE mutation for almost complete nakedness, resembling phenotypically the sex-linked naked[2] and autosomal scaleless[1] mutations in chickens, has arisen in a closed flock of Beltsville Small White turkeys. To the authors' knowledge no similar mutation has been previously described in turkeys.

The nakedness of these turkeys is considerably more extensive than it is in either scaleless[1] or naked chickens[2] (Figure 17). Feathering is confined to an average of 10 remigial feathers on the caudal margin of each wing and an occasional contour feather (down feather in the poult) on the elbow, wing web, shoulder, or breast. The rest of the body remains completely naked, with no evidence of either feather follicles or tracts. Histological preparations of skin biopsies from the capital pterylae of several naked poults reveal no feather follicles in a region which is feathered in a normal poult. The skin is smooth and translucent, resembling the apteria of a normally feathered turkey, except over the legs, shanks, and feet where it appears thicker and more opaque. The beak and toenails are normal, but there are no scales on the shanks and feet. The pads on the underside of the feet and toes appear thinner and more delicate than those of a normal turkey.

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Structural abnormalities of the legs and feet occur in nearly all naked poults, the commonest being the presence of one or more extra hind toes (with toenails) on one or both feet (Figure 18 and Table II). The location of the extra toe or toes varies from the level of the normal hind toe to a position in the upper half of the shank. The feet of many birds are flexed to such an extent that they are unable to stand. The legs, and particularly the ankle joints, of some are so twisted that the feet lie immobilized between the shoulders. If these birds were able to extend the legs they would project dorsally instead of ventrally. Often the head and neck are reflected posteriorly onto the midline of the back. General ataxia and weakness frequently prevent even those without structural defects from gaining and maintaining a standing position for any length of time.

It is possible to keep naked poults alive for a time without too much difficulty. One needs only to assure that they do not become chilled. This can be done by keeping them in a brooder until they become too large and then transferring them to an indoor pen with overhead infrared heating. By this method two such naked poults were raised to eight and 14 weeks of age, respectively. Both were sacrificed when perosis made it impossible to maintain them any longer. The first of these (a female) had feathers only on the caudal margins of the wings, and an extra toe on each foot. The second (a male) had two normal contour feathers on the right shoulder in addition to the usual wing feathers, and had an extra toe on the left foot (Figures 17 and 18).

FEET OF THE NAKED

Figure 18

The feet of a naked Beltsville Small White turkey are shown (see Figure 17). Note the extra hind toe on the left foot and the complete absence of scales on both shanks and feet. The left leg is severely affected with perosis.

Examination of breeding and pedigree records had previously indicated that some birds were carriers of the naked mutation and some were not. Test matings were made in 1958 and 1959 be-

TABLE I. Distribution of normally feathered and naked Beltsville Small White turkey poults and dead embryos from crosses between heterozygous naked and homozygous normally feathered females with heterozygous naked males

Year	Heterozygous naked dams			Homozygous normal dams		
	Normal	Naked	Total	Normal	Naked	Total
1958	1368	424	1792	1377	0	1377
1959	177	60	237	291	0	291

TABLE II. Sex ratio and frequency and type of polydactylism in naked F_1 poults from a cross between Beltsville Small White dams and sires heterozygous for the naked mutation (1960)

Sex of poults	No. exam.	Extra toe left foot	Extra toe right foot	Extra toe both feet	Multiple extra toes	No extra toes
Males	21	3	1	9	2	6
Females	22	3	1	10	0	8
Total	43	6	2	19	2	14

tween carrier males and carrier females, and carrier males and noncarrier females (Table I). The former produced normal and naked poults in approximately a three to one ratio, while the latter produced all normally feathered poults. Matings of carrier males and females in 1960 produced both male and female poults, in approximately equal numbers (Table II).

Three facts were evident from these data: (1) The carrier dams and sires in these matings were heterozygous for the naked mutation, (2) the mutation was inherited as a Mendelian recessive, and (3) the mutation was neither sex-linked nor sex-limited. The latter point demonstrated that this mutation was not homologous with the sex-linked naked mutation in chickens[2].

The authors propose the symbol *na* to designate this mutant character.

Whether the observed polydactylism is controlled by a gene separate from the naked mutation, or is merely a pleiotropic effect of the same gene, is not known at present. Forty-three naked poults were examined closely in 1960 for associated polydactylism (Table II). Twenty-nine of these poults had one or more extra toes while 14 had no extra toes. Examination of feathered sibs, numbering several hundred, yielded only one feathered poult with an extra toe on the left foot. These observations suggest that naked and polydactylism may be controlled by two separate, but closely linked genes. Whether or not this polydactylism is genetically homologous with polydactyly[4] and duplicate polydactyly[3] in chickens has not been investigated.

Summary

An autosomal recessive mutation for almost complete nakedness has arisen in Beltsville Small White turkeys. Feathering is confined to an average of 10 remigial feathers on the wings, and an occasional contour feather on the elbow, wing web, shoulder, or breast. Otherwise the skin is smooth and free of feather follicles. There are no scales on the shanks or feet. Most, but not all such birds have one or more extra hind toes on either one or both feet. Severe joint anomalies appear in the legs and feet of many.

The symbol *na* is proposed to designate this mutation. Matings of heterozygous adults produce homozygous naked poults in approximately a three to one ratio with no sex linkage or limitation. This mutation resembles phenotypically but is not genetically homologous with sex-linked naked and autosomal scaleless mutations in chickens. At present it is not known whether the associated polydactylism is a separate closely linked mutation or a pleiotropic effect of the *na* mutation. Homology with polydactyly and duplicate polydactyly in chickens has not been investigated.

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SOMBRE, A VIABLE DOMINANT MUTANT IN THE HOUSE MOUSE

NIGEL BATEMAN*

COMBINATION in one new mutant of three interesting properties of genes—mimicry, homology and unimpaired viability in homozygous dominants—compels this writer to describe the gene Sombre (symbol *So*).

Description

So/So mice are startlingly black, even inside and behind the ears and around the nipples. They fall short of being all black, however, through having a few yellowed hairs on the perineum. Only this feature spoils their perfect mimicry of extreme non-agouti (a^e/a^e)[1].

Although heterozygotes for Sombre also have dark ears and nipples, they are reminiscent of non-agoutis (a/a) in two respects. Often when non-agoutis are mature, their flanks are flecked with yellow hairs and their bellies are gray. The same expressions of maturity always occur in heterozygous Sombres where they are more conspicuous. Then both a/a and $So/+$ mice have considerable numbers of yellowed perineal hairs right from their first coats. For these reasons, homozygous and heterozygous Sombres need be seen only once together for each to be recognized infallibly in either sex from 12 days of age.

Genetics

The mutant phenotype first appeared with two normal littermates in the progeny of an ordinary sib mating within the C3H stock. A similar mouse was born three days later in an unrelated C3H family. This mouse had three normal littermates and died before weaning. In neither case was it possible to rear further litters as the sires had been discarded 16 days after mating, before the first litters were born. The two families had shared no common ancestors for nine generations, and a special environmental factor appeared to be implicated in the occurrence of the new phenotypes. It so happened that, in order to reduce the burden of ectoparasites, all 33 dams in the stock had been drenched with dibutylphthalate after the males were killed. Some dams were severely chilled by the treatment and fetal development was almost certainly affected in these. It was at first supposed that the new phenotypes were phenocopies, but this supposition was disproved on breeding.

When the surviving abnormal mouse was mated to an unrelated C3H male, her first litter contained three normal offspring and three "non-agoutis" like herself. Clearly, the new phenotype was in these animals the heterozygous expression of a mutant gene, now named Sombre (*So*). Since then, backcrosses of heterozygotes to normals have continued to yield equal numbers of Sombre and normal offspring (Table IA). There is a possibility of loose sex linkage because, when *So* was introduced through the paternal grandam, it tended to segregate with the X-chromosome, and conversely, segregated mostly with the Y-chromosome when derived from the paternal grandsire (Table IB i-ii).

The first indication that homozygotes survived was from the 3:1 ratio for the progeny of intercross matings (Table IC). Since then, lighter and darker versions of the *So* phenotypes have been recognized and shown to correspond to heterozygotes and homozygotes, respectively (Table ID i-iii).

The first interaction to be observed with other mutants was with White-bellied agouti (A^w). It was surprising to see in the F_1 that none of 10 heterozygotes for both *So* and A^w had lightened bellies; the only colored mutant previously known to suppress the light bellies of A^w was its allele A^y (Yellow). To test whether *So* was also an allele

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at the *A* locus, two of the Sombre/White-bellied agouti hybrids were backcrossed to normal mice, with results given in Table IE (i). Five Sombres and five normals appeared in first litters, the normals proving that Sombre and Agouti were not alleles. The absence of any White-bellied agoutis was a little disconcerting but, with the further litters that were raised, the backcross segregation eventually conformed to a 1:2:1 expectation for non-linked and epistatic loci. In its epistatic suppression of light bellies, *So* resembles the similarly autosomal dominant black gene (E^p) of the rabbit, albeit the rabbit gene is less completely dominant[2]. It will be interesting if recessive alleles of Sombre corresponding to e^j (Japanese brindled) and *e* (yellow) are discovered in the mouse.

No epistasis or allelism was shown in backcrosses involving brown (Table IE ii). *So* and *b* segregated apparently independently of one another but the segregation of $+^b$:*b* (5+5+1 stillborn : 12+10) was peculiarly unequal.

In backcrosses of albino/Sombre hybrids to albinos (Table IE iii) only three phenotypes appeared and roughly half the offspring were albino. The albinos clearly included both *c*/*c*; +/+ and *c*/*c*; *So*/+ genotypes, so that like all previous colored mutants, Sombre is hypostatic to albino. Again, no linkage is indicated.

Anomalous Segregation at Other Loci

Much larger numbers of backcross progeny were raised from the albino crosses than were required to determine

TABLE I. Segregation of *So*, and independence of sex, *a*, *b* and *c*

Text reference	Parents' genotypes	No. of matings	Offsprings' phenotypes			
A	*So*/+ × +/+	16	Sombre 102		Normal 108	
			Paternal combinations		New combinations	
B (i)	+X/+X × *So*X/+Y	2	Sombre female 25	Normal male 24	Normal female 18	Sombre male 24
(ii)	+X/+X × +X/*So*Y	9	Normal female 17	Sombre male 17	Sombre female 13	Normal male 14
		11	82		69	
C	*So*/+ × *So*/+	3	Sombre 34		Normal 13	
D (i)	*So*/+ (light *So*) × +/+	7	Dark Sombre 0	Light Sombre 26	Normal 29	
(ii)	*So*/*So* (dark *So*) × +/+	6	0	96	0	
(iii)	*So*/*So* (dark *So*) × *So*/*So* (dark *So*)	4	34	0	0	
			Parental combinations		New combinations	
E (i)	A^w+/+*So* × ++/++	2	A^w;+ 20	+;*So* 49 (Sombre)	A^w;*So* 21	+;+
(ii)	*b*+/+*So* × *b*+/*b*+	4	*b*;+ 12	+;*So* 5	*b*;*So* (chocolate) 10	+;+ 5
			17		15	
(iii)	*c*+/+*So* × *c*+/*c*+	18	+;*So* 52	*c*;+ 126 (albino)	*c*;*So* 53	+;+

the allelism and interaction of *c* and *So*. The reason was that the earlier data reinforced an impression of anomalous segregation already gained for A^w and *b*. In all cases, the heterozygotes ($A^w/+$, $+/b$ or $+/c$) were deficient in the backcross progeny, and especially in the first litters of heterozygous mothers. Segregation elsewhere would not have called for comment (Table II). The anomaly can hardly be attributed to fetal inviability. First, it is actually the heterozygotes that are deficient, although heterozygotes are usually gifted with superior viability; second, the perfectly normal proportion of Sombre mice appearing in the same material (Table III) does not permit the shortage of $+^b$ and $+^c$ animals to be attributed to an adverse effect on viability of chromatin from the Sombre stock. Hence if the results are to be explained otherwise than by remote chance, one must invoke the selective entry from the respective Sombre heterozygote of $+^b$ and $+^c$ into the nucleus of the egg. Yet it is difficult to foresee why this should be restricted to first litters. Investigation would not be called for unless the results recur, and no further work with the Sombre stock is contemplated in this laboratory.

Summary

Sombre (*So*), a new dominant mutant, is described in the mouse. Heterozygotes resemble non-agoutis, homozygotes resemble extreme non-agoutis; both appear to be fully viable and fertile. *So* is epistatic to A^w and is not allelic with *a*, *b* or *c*. Although the segregation of *So*: $+^{So}$ is perfect, peculiar segregation at these other loci has been noted in backcrosses.

TABLE II. Segregation in first and subsequent litters of three independent heterozygous loci from heterozygous Sombre dams and sires. (A^w:+ not scored in Sombres or before 12 days; *b*:+ and *c*:+ scored at birth.)

Genotype of segregating parent		Genotype of its mate		First litter offspring		Subsequent offspring	
				Homozygous recessive	Heterozygote	Homozygous recessive	Heterozygote
Sombre male	$+/A^w$	non-Sombre female	$+/+$	5	0	16	20
	$b/+$		b/b	11	9	—	—
	$c/+$		c/c	44	37	29	30
				60	46	45	50
				$P = 0.18$*		$P = 0.60$*	
Sombre female	$+/A^w$	non-Sombre male	$+/+$	—	—	—	—
	$b/+$		b/b	11	2	—	—
	$c/+$		c/c	41	24	17	21
				52	26	17	21
				$P = 0.005$*		$P = 0.50$*	

*P estimates overall probability of a 1:1 ratio.

TABLE III. Segregation of Sombre from heterozygous dams and sires in their first and subsequent litters. (*So*:+ not scored in albinos or before 12 days age.) Same material as in Table II.

Parent segregating Sombre	Other segregating mutants	First litter offspring		Subsequent offspring	
		Sombre	Normal	Sombre	Normal
Sire	A^w	5	5	44	36
	b	9	10	—	—
	c	15	19	15	12
		29	34	59	48
Dam	A^w	—	—	—	—
	b	6	7	—	—
	c	11	12	11	10
		17	19	11	10

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A BOGUS "SCIENCE" OF RACE PREJUDICE

RACE prejudice is one of the ugliest of human passions. This is, of course, a matter of personal opinion. At any rate, writings giving vent to passions do not belong on the pages of *The Journal of Heredity*, nor should they be taken notice of or criticized in this *Journal*. The situation changes however when such writings purport to be dealing with scientifically established facts, particularly facts of genetics and human biology. Silence should not be carried to the point of aiding and abetting misrepresentation. The book of Putnam* is a political tract combating the desegregation decision of the Supreme Court. The book carries, however, endorsements by Senators R. B. Russell, H. F. Byrd, and Strom Thurmond, of newspaper editor T. R. Waring, and finally by a quadrumvirate headed by none other than R. Ruggles Gates, styled as "generally acknowledged to be one of the world's leading human geneticists," and including a presumably equally leading psychologist, anthropologist, and anatomist. The book is claimed to be a "balanced presentation of genetic and environmental factors in the area of both racial and individual biology."

A presentation of such "factors" would be of interest to the readers of *The Journal of Heredity*. However, the book has no such presentation, either balanced or unbalanced. It is perfectly obvious to the author that differences between people are hereditary, since "Intelligence is almost entirely a matter of heredity. Heredity is also substantially involved in character—ask any man who knows and loves animals—but it is more subject to modification by environment than intelligence." It is no less evident that "There is no basis in sound science for the assumption, promoted by various minority groups in recent decades, that all races are biologically equal in their capacity to advance, or even to sustain, what is commonly called Western civilization. They most emphatically are not." And of course, "The white man who preaches to backward races a doctrine of equality not only demeans himself and his own race, but forfeits his opportunity to be of real service. What is called the 'liberal ferment' among backward peoples who are shouting democracy from Latin America to Africa is too often not at all a struggle for freedom under law on the part of peoples capable of self-government, as was the case in the American Revolution, but rather a demand for license under lawlessness on the part of peoples totally incapable of self-government." Variations of these pronouncements recur again and again, perhaps to make them more "scientific" by repetition.

The nearest it ever comes to adducing evidence is, curiously enough, in the form of nine "questions." Thus: "1. Can you name one case in all history in which whites and Negroes in large numbers have lived together without segregation and have failed to intermarry? 2. Can you name one case in all history in which a white civilization failed to deteriorate after intermarrying with Negroes? 3. Can you name one case in all history of a stable, free civilization that was predominantly, or even substantially, Negro?" And so forth. The author knows that the meaning of such questions, and of answers to them, has been clarified by modern anthropology, and he has a lot to say about this. Why, it's all a conspiracy! "I would go so far as to say that in the last fifty years anthropology has been drafted to serve the demi-goddess of Equalitarianism instead of the goddess of Truth, and that the modern school in this field has a stern judgment to face, both at the bar of American public opinion and at the hands of two generations of youth whose thinking has been corrupted by it." The master mind of the conspiracy turns out to be Boas, ". . . a foreign-born Columbia University professor who arrived in the United States in 1886, who was himself a member of a racial minority group, and who may be called the father of equalitarian anthropology in America." Boas's "racial minority" is not named; it was Jewish. And so, Boas has started this ". . . clever and insidious prop-

**Race and Reason, a Yankee View.* Carleton Putnam. X + 126 pp. Public Affairs Press, Washington. 1961.

aganda posing in the name of science, fruitless efforts at proof of unprovable theories, which I would be only too glad to point out to the Attorney General." It was Boas who started the school—Herskovits, Klineberg, Ashley Montagu—which "victimized" two generations of Americans ". . . by a pseudo-scientific hoax in this field, . . . part of an equalitarian propaganda typical of the left-wing overdrift of our time." To be sure, there were some equally bad non-racial-minority anthropologists: "I have found that a favorite method used by Boas and Kluckhohn for throwing dust in the eyes of the public is to create an impression that there is really no such thing as race."

The adjective "scientific" has nowadays become invested with a kind of sacred *mystique*. It means different things to different people, but by no stretch of its meaning can Putnam's book be said to be "scientific." Race prejudice arises from emotion, not from reasoning, although time and again it sought to shore itself up with pseudo-science. This was most notably true in Hitler's Germany, and because of this association with Nazism this pseudo-science fell temporarily into desuetude in most of the world. It was to be expected that the murky tide will stage a comeback, and this is what we are actually observing.

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Addendum: While this review was in the press, the reviewer received a copy of Putnam's pamphlet accompanied by a note, signed by Senator Harry F. Byrd of Virginia, saying in part: "Because I believe this book to be of exceptional importance, I urge it upon your immediate attention. It is sent to you with the compliments of the North-South Council, out of funds contributed by people in both the North and the South." It seems that geneticists and anthropologists may well give their immediate attention to the danger of misuse of their sciences for propagandistic ends.

Heredity and Human Nature

THE purpose of this book* on human behavior genetics is to introduce the reader to certain basic differences among populations, and to present factual information on the underlying causes for these variations. In exploring individual and group differences in various areas of behavior, the author makes a great effort toward evaluating the effect of innate inequalities on human relations. His contention is that appreciation and understanding of the interplay between the sociological and biological determinants of man's nature are prerequisites for effective management of the interpersonal and international problems that beset mankind. While emphasis is placed on the desire of a democratic society to treat all individuals equally, the salient point of the book is that populations evolve with unequal inherited endowments.

Throughout the nine chapters of the book, information is presented on genetic differences among individuals in health and disorder, although the main theme is established in discussing trait variations in relation to race, religion, and ethnic origin. Some of the data on which these discussions are based fail to meet strict, modern-day scientific criteria, and it would definitely be helpful if the author's plea for more research on the genetics of human behavior were not to go unheeded. The newcomer to a scientific discipline may find it rather difficult, without expert guidance, to evaluate either suggested source material or some of the information presented in the text.

While this book has shortcomings, it should be of interest to a behavioral scientist as a challenging and thought-provoking treatise in some controversial areas of human genetics.

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**Heredity and Human Nature.* David C. Rife. Vantage Press, New York. 1959. pp. 265. $4.50.

AN OCTOPLOID POTATO FROM EYE-EXCISION EXPERIMENTS

H. W. HOWARD*

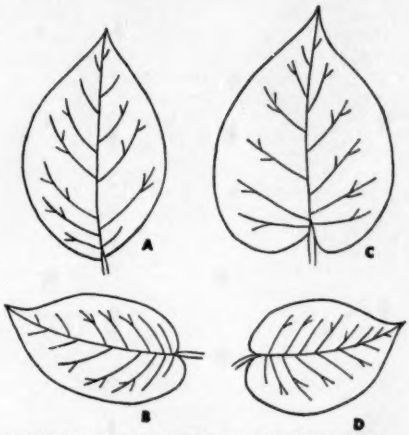
THE METHOD of removing all the eyes from potato tubers so that adventitious bud formation takes place from the inner tissues has been widely used in investigating periclinal chimeras[1,5,9]. In other plants, e.g. *Brassica oleracea*[4], *Lycopersicon esculentum*[8], and *Nicotiana* species[3], it has been found that some adventitious buds formed from inner tissues give rise to plants with a doubled chromosome number. There does not appear, however, to be in eye-excision experiments with potato tubers any record of the production of plants with a doubled chromosome number.

Materials and Methods

Eyes were removed from five half-tubers (split longitudinally) of the variety Arran Victory, and the treated half-tubers were placed on moist peat in dishes for three weeks to ensure that all buds in the eyes had been removed. The half-tubers were then planted in pots, and three of them eventually gave rise to plants. One of these plants was seen to be peculiar in that it had very broad leaflets (Figure 19). The control half-tuber, from which the eyes had not been removed, produced a normal plant. Three tubers were formed by the plant with broad leaflets, and these were grown on in the following year. All three plants were similar to their parent in having broad leaflets.

Octoploid Arran Victory

Normal Arran Victory, like other varieties of *Solanum tuberosum*, is a tetraploid with a chromosome number of $2n = 48$. Root-tips from the plants with broad leaflets were found to be octoploids with about 96 chromosomes. The counts were made on squashes of tips placed in 8-hydroxyquinoline overnight and then stained by the Feulgen method.



BROAD LEAFLETS IN OCTOPLOIDS

Figure 19

Terminal (*A*) and first lateral (*B*) leaflets of normal Arran Victory; terminal (*C*) and first lateral (*D*) leaflets of octoploid Arran Victory. (2/3×)

The increased leaflet width (breadth/length ratio of 0.78 for terminal leaflets as compared with 0.64 for normal Arran Victory) agrees well with similar data for potato species and hybrids doubled by colchicine treatment[7]. The octoploids also have larger stomata (average size $49\mu \times 31\mu$) than those of normal Arran Victory (average size $36\mu \times 28\mu$). Other features shown by the octoploids were thicker stems, reduced height, and a marked reduction in the number of leaflets per leaf. Also, unlike normal Arran Victory, they failed to flower. Their tubers were similar to normal Arran Victory in being a deep purple color.

Discussion

It would appear from the stomata and leaflet shape measurements and from the root-tip chromosome count that the octoploid plants are entirely octoploid and are not a tetraploid-octoploid chimera. This suggests that the callus bud which gave rise to the original

*Plant Breeding Institute, Cambridge, England.

octoploid plant could be traced back to a single octoploid cell formed during the production of the wound cambium or at the most to a small piece of octoploid tissue in the half-tuber. Such a suggestion is in agreement with the results of eye-excision experiments with periclinal chimeras in that the plants produced by this treatment are not themselves chimeras.

Although in the Arran Victory eye-excision experiment, one out of the three half-tubers which grew produced an octoploid plant, the percentage of octoploids formed by eye-excised tubers is probably less than one percent. Thus no octoploids have been found in the experiments with Red King[6] nor in the experiments with white-splashed-color tubers[6]. It is also unlikely that they have been missed by other workers because the broad leaflets and other characters of octoploid potatoes are easily identified.

Howard[4] has discussed briefly the possible ways in which adventitious buds with doubled chromosome number could be formed by or near callus tissue produced after wounding. He concluded that the most probable cause was by the division of vacuolated cells which had nuclei with the doubled chromosome number and which, except for the stimulus produced by wounding, would never divide. Some support for this explanation is given by the work of Grafl[2] on tubers of *Sauromatum guttatum*. In tubers of this plant, cells with $4x$ and $8x$ nuclei were observed in normal tissues, but below wound surfaces large 16-ploid nuclei were found.

Summary

An octoploid ($2n = 96$) plant of the potato variety Arran Victory was obtained in an eye-excision experiment. The octoploid was first recognized by leaflets much broader than those of the tetraploid parent.

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